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THE ESTIMATION OF SMALL QUANTITIES
OF FERMENTABLE SUGARS BY CARBON
DIOXIDE PRODUCTION

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(With 2 figures in the text)

IN the estimation of sugars in complex mixtures, such as occur in plant extracts, fermentation methods have been found indispensable and are used by all workers. The procedure commonly adopted is to ferment away the sugar mixture completely and to estimate the loss of ability to reduce alkaline cupric copper, ferricyanide, etc. The residual reduction is regarded as being due to non-sugars and is deducted from the initial reduction determined before fermentation to correct the estimate of fermentable sugars present. Such a method is satisfactory allowing two assumptions. In the first place it must be assumed that the yeast destroys only sugars among the suitable reducing substances present, e.g. there must not be appreciable quantities of fermentable phosphoric esters, glycer-aldehyde, etc., in the mixture. In the second place the yeast must not give rise during the removal of the sugars to substances capable of reducing copper or ferricyanide.

It is usually safe with plant extracts to assume that the sugars present will greatly outweigh the other fermentable substances, and errors due to the first assumption are not likely to be important. The second assumption may be more troublesome, particularly if the "unfermentable reducing power" is an appreciable fraction—say 20 per cent. or more—of the whole. There is usually no means of telling how much of this fraction was present at the start and how much has arisen during the period of fermentation. In the course of some experiments on barley embryos this difficulty was met in an acute form. After 3 hours' fermentation with bakers' yeast (in an

air stream to avoid the formation of alcohol) the reducing power of the extracts, so far from disappearing completely, was actually considerably greater than at the start. The following results were obtained for the reduction of alkaline ferricyanide after the method of Hagedorn and Jensen.

TABLE I. Reducing power of extract from 100 embryos, c.c. N/100 thiosulphate in the Hagedorn-Jensen titration

Before fermentation	After fermentation	Increase
6.43	10.70	+4.27
6.53	11.47	+4.94

Such results can only be due to the formation of suitable reducing substances by the yeast in excess of any sugars simultaneously destroyed.

In the present instance there is a strong possibility that the substance formed is acetyl methyl carbinol ("acetoin"), $\text{CH}_3\text{CHOH.CO.CH}_3$. It is already known to be formed by yeast, especially when strongly aerated, as is necessary in the above experiments to avoid the formation of alcohol (Elion, 1927). It reduces Fehling's solution strongly. An attempt was made to detect the appearance of new carbonyl groups, which would necessarily happen if acetoin was formed, by measuring the bisulphite binding capacity of the solution before and after fermentation with the following result.

TABLE II. Mg. bisulphite bound by extract from 100 embryos, c.c. N/100 iodine \equiv bisulphite bound

Before fermentation	After fermentation	Increase
4.85	9.37	4.52

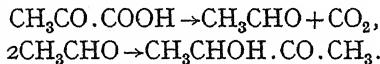
This increase if due to acetoin is more than would be required to account for the simultaneous increase of reducing power, as is to be expected if some sugar is being destroyed at the same time.

Negative results with the "dinedon" test (Simon and Neuberg, 1932) and Schiff's reagent also suggested that the bisulphite compound might be formed by a ketone rather than an aldehyde. As a specific test for the presence of acetoin the usual method of oxidation to diacetyl, distillation of the diacetyl and subsequent formation of the nickel dimethylglyoxime (Kluyver, Donker and Visser 't Hooft, 1925) was employed; this test is very sensitive. A slight reaction indicated by a blue coloration was obtained with the extract before fermentation, agreeing with the result of Lemoigne and Mongouillon (1930) who have previously shown that acetoin is

Estimation of Fermentable Sugars by Carbon Dioxide 3

formed during the germination of barley grains. With the extracts after fermentation a much stronger reaction (a red precipitate) was developed.

Table I shows that one or more substances able to reduce actively in alkaline solutions were formed during the fermentation of the extracts. This makes the combination of reduction and fermentation methods unsatisfactory with these and perhaps other plant materials. There seems to be no alternative reagent to yeast for picking out fermentable sugars, but the difficulty might be avoided by estimating the carbon dioxide evolved during the fermentation instead of the change of reducing power. Several advantages might be expected in theory. (1) The results would be independent of other reducing substances present; (2) there would be no objection to the formation of alcohol in moderate quantities, and hence the use of aerobic conditions and the consequent risk of intrusive oxidative processes could be avoided; (3) acetoin even if formed does not influence the carbon dioxide output since decarboxylation occurs at an earlier stage of the reactions, the following equations having a high degree of probability, as representing the changes brought about by yeast:



It has also been shown directly that the presence of acetoin does not influence the first reaction (Neuberg and Kobel, 1925). The following section gives the details of a method we have worked out for small quantities of sugars based on these principles.

MATERIALS USED

(1) *Yeast.* A fresh supply of pressed bakers' yeast is obtained daily or a stock may be kept for a few days in an ice-chest. 10 gm. are weighed out, washed with distilled water and thrown down in a centrifuge; a single washing is sufficient. After decanting off the washings fresh water is added, the yeast stirred into a suspension and made up in a measuring flask to 100 c.c.

(2) *N/100 HCN.* This is prepared by weighing out pure potassium cyanide, dissolving and adding 50 per cent. hydrochloric acid until a small piece of litmus paper included in the solution becomes purple red. The solution is then made up to the required volume and may be kept as stock more or less indefinitely.

(3) *Nitrogen.* Commercial compressed nitrogen is used. This contains about 1 per cent. of oxygen. To remove this is troublesome,

and it would also be difficult to avoid the introduction of traces of oxygen from the outside air during manipulation. These precautions become unnecessary when fermentation is carried out by the technique described below.

(4) *Baryta solution.* This is made up at approximately N/150 by the usual methods.

(5) *Standardised hydrochloric acid.* A stock of approximately normal hydrochloric acid is prepared and diluted two hundred times. The roughly N/200 acid is standardised accurately as follows. An accurate N/100 sodium carbonate solution is prepared by weighing out oven-dried (or ignited) anhydrous sodium carbonate (we used B.D.H. "analar"). 5 c.c. are measured accurately into a boiling tube and a little brom thymol blue added as indicator. The solution is raised to boiling point over a small flame and the hydrochloric acid run in cautiously from a microburette. The carbon dioxide evolved is boiled off and further hydrochloric acid run in until the colour after boiling indicates pH 7·0. When the end-point is approached the indicator colour is tested against a standard in a comparator. If necessary further additions of acid are then made. Owing to the nature and dilution of the reagents accurate titration to pH 7·0 is essential. Once the strength of the acid has been determined in this way, the baryta solution can be titrated against it, and it is more convenient to standardise subsequent batches of acid against the known baryta, provided that this is kept secure from atmospheric carbon dioxide.

(6) 1 per cent. alcoholic phenolphthalein as indicator.

THE FERMENTATION

Before the estimation proper the yeast is shaken in a bath (about 50 oscillations per minute) at 35° C. for 1 hour. 1 c.c. aliquots of the yeast suspension (p. 3) ≡ 100 mg. of yeast are transferred to fermentation tubes of the form shown in Fig. 1. 3 c.c. of distilled water and 1 c.c. N/100 HCN are added, making a total of 5 c.c. in the tube. The preliminary starvation of 1 hour serves to reduce the rate of spontaneous carbon dioxide formation ("autofermentation") to a half or less of its initial value. It is naturally desirable that the amount of carbon dioxide due to this cause should be small relative to the amount produced from the added sugar. Another advantage is the adjustment of the suspension to the standard temperature before the introduction of the sugar.

When the yeast suspension has been starved for an hour it is

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ready for use. The tube containing it is removed from the bath (p. 4) and exhausted by attaching the outlet *B* to a filter pump *via* the exhaustion chamber, Fig. 2, *E*. This removes the carbon dioxide now present in the fermentation tube, and the last traces are swept out by closing the clip *e* and allowing nitrogen to enter from the aspirator by opening the clip *a*, until the pressure in *E* returns to the

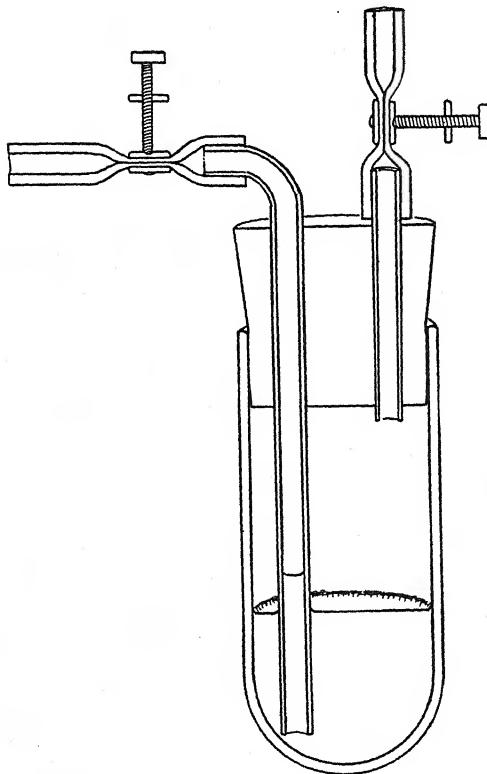


Fig. 1.

atmospheric. *E* should be about 350–400 c.c. capacity (=7 or 8 times that of *F*). The tube is now detached from *E* and nitrogen allowed to flow gently through it while 1 c.c. *N*/100 HCN is run in through *B* followed by a known volume, 2 or 3 c.c., of the sugar solution and distilled water to make a total volume of 10 c.c. including the 5 c.c. already in the tube. Care must be taken that the solutions are introduced cleanly into the cavity of the fermentation tube, and for this a burette with a lengthened narrow jet is very useful. The

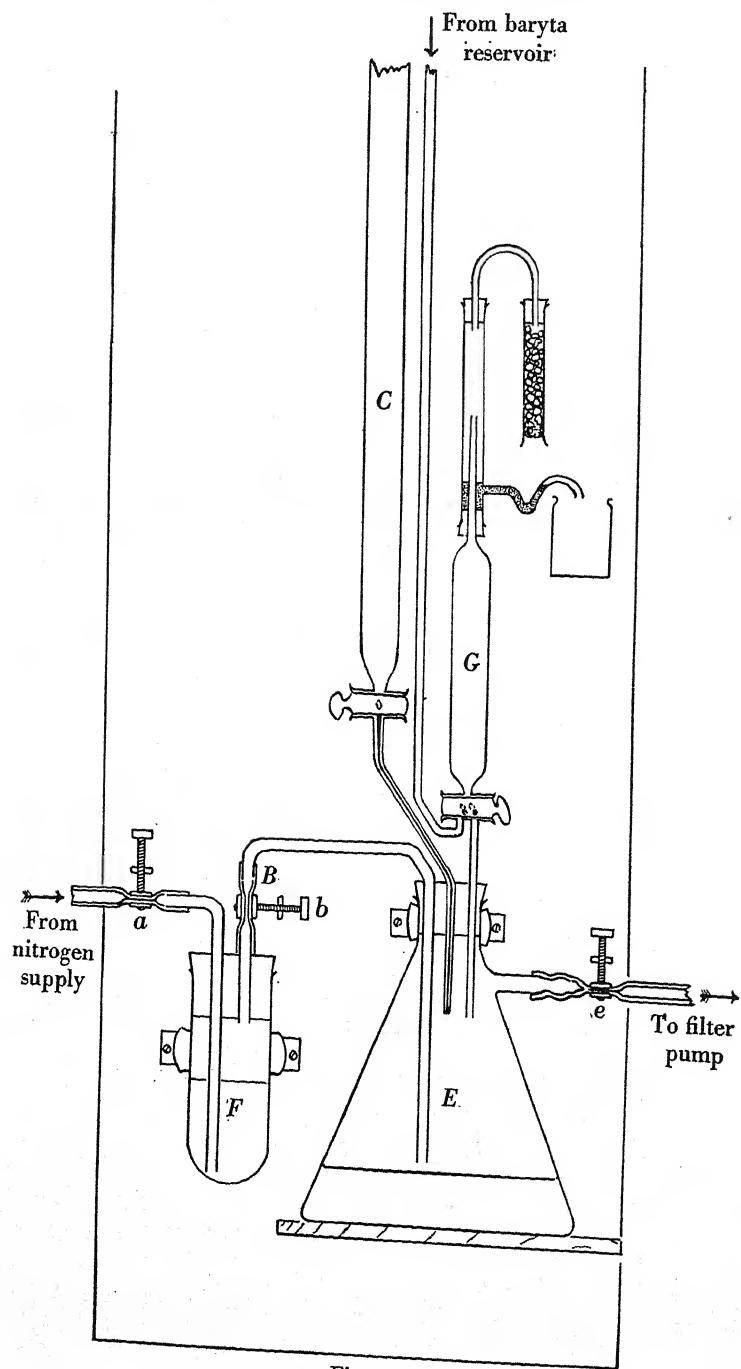


Fig. 2.

Estimation of Fermentable Sugars by Carbon Dioxide 7

burette is then withdrawn slowly, the clips *a* and *b* immediately closed and the fermentation tube put back into the water bath at 35° C. and shaken for 2 hours. The total volume of liquid in the tube should now be 10 c.c. and will be made up as follows:

- 1 c.c. yeast suspension (\equiv 100 mg. yeast).
- 2 c.c. N/100 HCN (final concentration = N/500 HCN).
- x* c.c. sugar solution.
- 10 - (*x* + 3) c.c. distilled water.

When a number of experiments are to be performed consecutively it is wise to have 15–20 min. intervals between starting one tube and the next.

METHOD OF TITRATION

The carbon dioxide formed in the fermentation tube is estimated by precipitation with baryta and titration of the residual alkali; the estimation is carried out in a carbon dioxide free atmosphere in the apparatus shown in Fig. 2.

The fermentation tube is connected up as shown and the chamber *E* exhausted while the clip *b* is still shut. In setting up *B* a few drops of phenolphthalein are included and 25 c.c. of baryta solution are run in from the automatic pipette (*G*) while exhaustion is in progress. Clip *e* is then shut and the carbon dioxide evolved in *F* is drawn over by opening *b* and then *a* so that carbon dioxide free nitrogen flows into *E* through the liquid in the fermentation tube (*F*). The whole of the carbon dioxide in *F* including that in solution is thus swept over into *E* (see p. 8). The whole apparatus is mounted on a board hinged to a stand at the top and free at the bottom. This is necessary so that the baryta solution in *E* can now be well shaken up to absorb the carbon dioxide above it. A simple mechanical shaking device is desirable. Five minutes' fairly vigorous shaking is enough. The residual baryta is now titrated with the standard acid run in from the burette *C*. The carbon dioxide value is obtained by deducting the acid equivalent of the residual baryta so obtained from the corresponding value for the full amount of baryta. From this the equivalent weight of sugar may be simply calculated relating carbon dioxide to hexose sugar by the fermentation equation

$$1 \text{ c.c. } N/200 \text{ HCl} \equiv 0.225 \text{ mg. } C_6H_{12}O_6.$$

TESTS OF THE METHOD

Estimation of carbon dioxide. The accuracy of the carrying over and absorption of the carbon dioxide was tested as follows. 2 c.c. of standard N/10 sodium carbonate solution were put into a fermentation tube. An excess of phosphoric (or sulphuric) acid was put into a small tube which was gently inserted. The stopper was then put in and the carbon dioxide liberated from the carbonate by spilling the strong acid into it. When the reaction was complete the carbon dioxide was drawn over and shaken up with the baryta in the usual way.

TABLE III

CO ₂ in 2 c.c. Na ₂ CO ₃	calculated			
"	estimated	15 min. shaking	4·40 mg.	
"	"	15 "	4·38 "	
"	"	10 "	4·43 "	
"	"	5 "	4·41 "	
			4·39 "	

The error of the estimation in every case is less than 1 per cent.

Correction for carbon dioxide of autofermentation. Estimations of the carbon dioxide given off without the addition of external sugars were carried out with several batches of yeast at various times. The mean value of these was used as a deduction from all experimental values. This procedure is less laborious than carrying out a control value with every experiment. Applying the latter method did not lead to any increase of accuracy; rather the reverse indeed, since the individual values recorded for autofermentation in the earlier experiments were less regular than those with added sugars. With practice the irregularity disappeared. The values obtained (calculated as equivalent hexose) were 0·46, 0·04, 0·26, 0·24, 0·34, 0·26, 0·27. Average 0·27 mg.

The use of HCN. According to Meyerhof (1925) HCN in concentrations between N/500 and N/1000 almost entirely suppresses oxidative effects in bakers' yeast without markedly retarding the rate of fermentation. This applies with the normal atmospheric percentage of oxygen. Using N/300 HCN Dixon and Elliott (1929) found that inhibition in the presence of sugars was 92 per cent., but only about 85 per cent. for autofermentation even with the cyanide 10 times as strong. In our own experiments using N/500 HCN the CO₂ yield in air was only 82 per cent. of the theoretical, but with 1 per cent. oxygen (commercial "nitrogen") the yield in a companion experiment rose to 98·8 per cent. Experiments with varying concentrations of HCN gave the following results (Table IV).

Estimation of Fermentable Sugars by Carbon Dioxide 9

TABLE IV. Fermentation in nitrogen containing 1 per cent. of oxygen.
Yields of carbon dioxide as percentages of the theoretical, mg. sugar supplied

HCN	0·96	1·92	2·88	3·84	4·80	5·79
N/1000	57	64	77	—	69	—
N/700	—	79	—	—	—	79
N/500	117	103·5	98·8	98·9	99·0	90·0
N/250	—	—	80-85	—	—	90

Approximate percentages only are given for concentrations of HCN other than N/500, as but few autofermentation readings were taken.

The results show that with quantities up to 5 mg. of sugar good estimations may be made using N/500 HCN. At the lower concentrations, e.g. with about 1 mg. sugar, the error is still small but naturally increases as a percentage. The most satisfactory range lies between 3 and 5 mg., where the percentage error is about 1. With quantities greater than 5 mg. low results are recorded, and longer fermentation times or more yeast would be required.

Example of an estimation. The fermentation tube contained:

1 c.c. yeast suspension (\equiv 100 mg. yeast).

2 c.c. N/100 HCN.

4 c.c. glucose solution (\equiv 3·84 mg. glucose).

3 c.c. distilled water.

10 c.c.

Titration: 25 c.c. baryta \equiv 47·2 c.c. HCl (0·060 N).

After absorption \equiv 32·30 c.c. HCl.

$\therefore \text{CO}_2$ \equiv 14·90 c.c. HCl.

$= 14·90 \times 0·272$ mg. glucose.

4·05 mg. glucose.

0·27 mg. glucose: autofermentation correction.

3·78 mg. glucose fermented = 98·44 per cent.

Estimation of sucrose and maltose. These disaccharides are fermented by bakers' yeast, but using the technique described above very low values were obtained.

To obtain complete fermentation more yeast would therefore be required. This would inevitably raise the autofermentation value to a considerable percentage of the whole CO₂ output. It is, therefore, considered better to hydrolyse the disaccharide first and estimate it in the hexose form.

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VERTICAL RESIN DUCTS IN THE SECONDARY WOOD OF THE ABIETINEAE

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(With Plate I and 23 figures in the text)

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INTRODUCTION

ALTHOUGH the resin ducts in the Abietineae have been the subject of much investigation, the studies in the main have lacked the comprehensiveness necessary for phylogenetic evaluations. Certain features have been described more or less completely for a number of forms, and more thorough investigations have been made of particular characters in certain species, but studies of the latter type, expanded to include a number of genera, have been attempted in few cases. It was with a view to filling in some of these gaps, in order to provide a more complete basis of comparison, that the following investigations were undertaken.

It has been shown that in such conifers as *Abies*, *Tsuga*, *Cedrus* and *Pseudolarix* vertical resin ducts are absent from the secondary xylem, except at injuries where they occur in tangential series. In *Larix*, *Pseudotsuga*, *Picea* and *Pinus*, on the other hand, the ducts are often widely scattered and have been considered to be a more or less normal feature of the xylem. On these differences in distribution Jeffrey (1905) divided the Abietineae into two subtribes,

Abies, *Tsuga*, *Cedrus* and *Pseudolarix* composing the Abieteae, and *Larix*, *Pseudotsuga*, *Picea* and *Pinus* the Pineae.

In the Abieteae the ducts are sac-like, and are either isolated or branch and fuse with others in the series to form an anastomosing network of cavities. The ducts in the Pineae are often farther separated, and unite with others at fewer points, so that the shape of the duct more closely approximates to that of a tube or canal. These differences were recognised by Penhallow (1907), who applied the term "cyst" to the ducts in the Abieteae and "canal" to the scattered, tube-like ducts in the Pineae. This terminology will be adhered to in the following descriptions when reference is being made to that particular character. Otherwise the more general term "duct" will be used.

ORIGIN OF THE RESIN DUCTS AND ASSOCIATED TISSUE

Mayr (1884), from his study of *Larix* and *Picea*, concluded that certain mother cells soon after their origin from the cambium were segmented into a number of shorter cells, some of which in turn were divided by longitudinal walls. In this mass of tissue the duct arose by schizogeny, the cells around the duct forming a parenchymatous epithelium and the more distant ones becoming either parenchymatous or tracheary. Some time later Kirsch (1911) proposed a different type of origin. He suggested that the cells surrounding the duct arose, not from fusiform cambial initials, but by proliferation from the xylem rays.

The origin of the tissue surrounding the resin passages may be determined either by examination of the shape and arrangement of the cells in the mature xylem or by study of the developmental stages. For the first of these methods transverse, radial and tangential sections may be utilised. In transverse sections of any of the Abietineae, except possibly *Pinus*, it may be seen that the cells surrounding the duct lie in the same horizontal rows as the tracheids immediately preceding and succeeding the tissue in question. Similarly, in radial sections careful examination shows that the short parenchyma cells are in longitudinal groups, and that the upper- and lowermost cells of each group are in the same horizontal planes as the ends of the adjacent tracheids produced before and after the parenchyma cells (Text-fig. 11). Furthermore, in tangential section the fusiform outline of these vertical groups of parenchyma cells is readily discernible in the majority of cases. The results obtained from the study of sections cut in these three different planes agree in

demonstrating that the parenchymatous tissue arises by segmentation of fusiform elements.

In the case of *Pinus* it is more difficult to determine satisfactorily the relationships of the parenchymatous cells surrounding the mature duct, owing to the fact that the majority remain thin-walled and consequently are distorted and displaced to a greater degree by the enlargement of the duct. But even here, as Hart (1916) has shown, the fusiform groupings of the cells are discernible in many instances. Further, when sections are cut through the newly formed tissues in the vicinity of the cambium, before the duct has widened, the fusiform outlines of the groups of parenchyma cells may be clearly seen.

An examination of the developmental stages confirms the conclusions arrived at from the studies of the tissue surrounding the mature duct. The steps in the development of the duct and its encompassing cells, as seen in a tangential section cut through the recently formed tissue in the vicinity of the cambium, are represented in Text-fig. 1. This diagram illustrates the structures in a section cut at a slight angle to the vertical plane, so that the top passes very close to the cambium proper and the bottom through the recently formed xylem. In the upper part the early stages in the segmentation of the fusiform elements may be seen, and in the lower part the final stages of this segmentation and the first appearance of the duct. In the case of those fusiform elements which will form the investing layer or epithelium of the duct, segmentation proceeds rapidly and large numbers of short cells are formed. Some of these may in turn be divided by vertical walls in radial or less often in tangential planes, but such division is sporadic and varies greatly in different specimens. The elements more distant from the duct are divided by fewer transverse walls and the daughter cells are considerably longer.

The evidence derived from the studies of the tissue both at the developing and the mature duct shows that the cells are formed by the segmentation of fusiform elements, the origins of which are the fusiform initials in the cambium. No support is to be found for Kirsch's theory that these cells arise by proliferation from the xylem rays. Associations of rays and resin ducts, as described and figured by Kirsch, do occur, but in these instances it is a case of rays arising at the resin tissue, rather than of resin tissue being proliferated vertically from the rays. The vertically elongated shape of the ray cells in contact with the parenchyma cells at the duct, which

Kirsch considered evidence of proliferation in the vertical direction, is apparently due to the recent origin of the rays. It has been shown elsewhere (Bannan, 1934) that the first-formed cells of rays arising in the wood near the pith (the type of wood used by Kirsch) are of vertically extended shape, and this holds regardless of whether the rays begin at resin ducts or in the normal wood quite apart from the ducts. The shape of the ray cells is correlated with the origin of the ray, and is not a demonstration of vertical proliferation.

The duct arises by a schizogenous separation of the cells in the centre of the mass of parenchyma tissue (Text-fig. 1). Very little evidence of lysigeny was found in any of the genera studied. There is a forcing apart of the cells, and in some cases striking illustrations are found of the stretching of the cells across the duct when they have not entirely separated from one another but have maintained contact at one or more points (see Text-fig. 8). The forcing apart appears to be brought about by the accumulation of resin in the duct. Hanes (1927) has mentioned this as the probable mode of origin in the case of the primary resin canals. The resin, which is recognisable in the ducts at a very early stage, was believed by Tschirch (1906) to arise in a resinogenous layer in the duct, but Hannig (1922) and Franck (1923) have found resin in the epithelial cells exhibiting the same reaction to microchemical tests as that in the duct. According to Franck such resin is demonstrable in these cells even before the inception of schizogeny, and both Franck and Tschirch have observed resin in the duct during the earliest stages of its enlargement. The duct widens rapidly and in many cases reaches its ultimate size before the walls of the tracheids on either side are completely thickened and lignified.

CHARACTER OF THE TISSUE ASSOCIATED WITH THE DUCTS

The resin ducts in the Abietineae are typically enclosed by a ring of parenchyma cells termed the epithelium. Surrounding the epithelium there are usually other parenchyma cells, which sometimes form a complete ring and in other cases occur only at intervals. These outer parenchyma cells in turn are generally enclosed by elements of fusiform outline, the segments of which may be in part parenchymatous and in part tracheary, or totally tracheary. The septate tracheids or tracheids adjacent to the parenchyma cells commonly have smaller pits than the more distant tracheids (Text-figs. 2 and 11). The tracheary elements associated with the ducts will not be further described, but since there is considerable variation

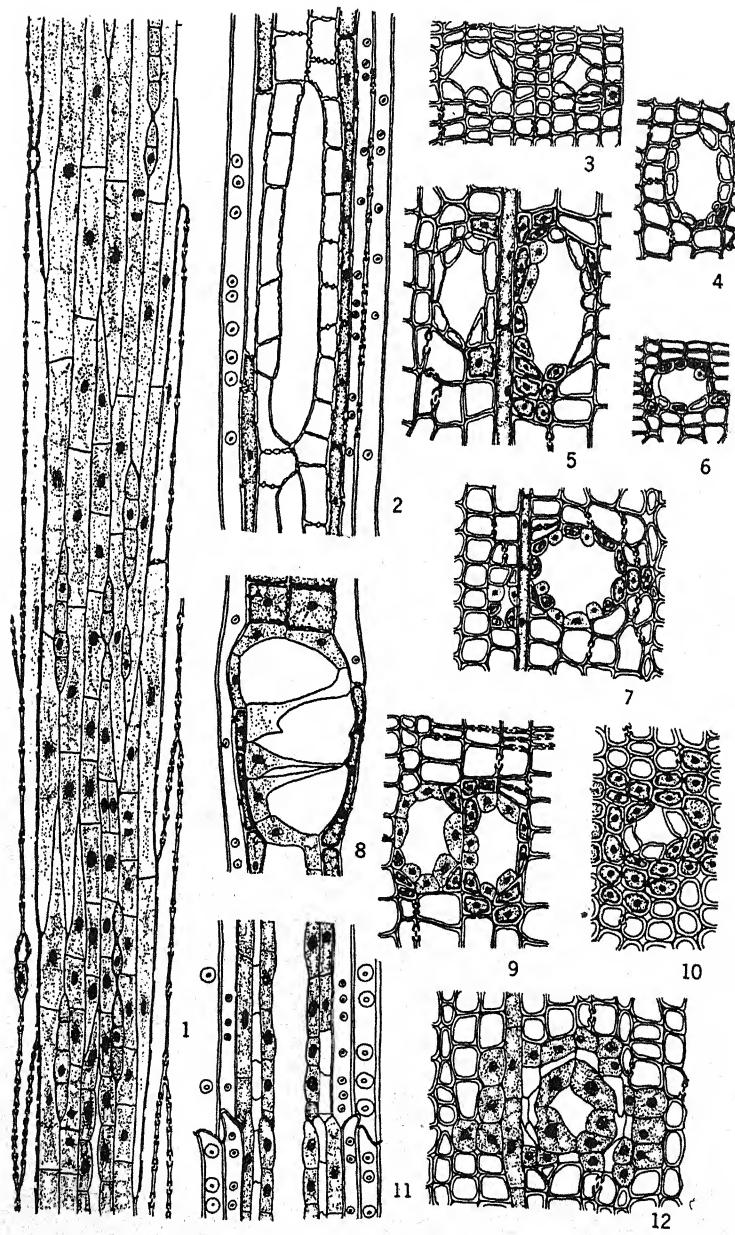
in the character of the epithelium and surrounding parenchyma cells in the different genera, these cells will be more fully dealt with.

In *Abies* and *Tsuga* the epithelial cells become thick-walled and lignified soon after their origin, and the protoplasm disintegrates (Text-fig. 3). Only rarely do these cells remain thin-walled and unlignified, and in few cases does their plasm function beyond the first season. The surrounding parenchyma cells, like the epithelium, soon become thick-walled and lignified. But in contrast to the latter, many of the surrounding cells retain their protoplasm for several years (Text-fig. 2), though the number that do and the length of time that the plasm functions varies widely.

The tissue surrounding the ducts in *Pseudolarix* resembles that in *Abies* or *Tsuga* except that many of the epithelial cells retain their plasm for longer periods of time and in this respect more closely resemble the outer parenchyma cells. Likewise in *Cedrus* and *Keteleeria* quite a large number of the epithelial cells remain active beyond the first year. The walls of the majority of these cells are thickened and lignified shortly after the cell origin, the usual procedure in *Abies* and *Tsuga*, but in other cases the walls remain thin and unlignified for a number of years. In the available specimens of stem wood of *Cedrus* from 15 to 30 per cent. of the epithelial cells were of this thin-walled type, and although the proportion in *Keteleeria* was not accurately determined it also was quite large.

The tissue associated with the ducts in such Pineae as *Larix*, *Picea* and *Pseudotsuga* has certain points of similarity with that in the Abietineae, but there is considerable variation both between the three genera and different specimens of the same species. In some cases the walls of the epithelial cells are thickened and lignified soon after the cell origin. The cytoplasm may disintegrate during the same year, as in *Abies* and *Tsuga*, or persist for several years. Other cells remain thin-walled until the close of the first season, or perhaps into the second or third years, before the wall lignifies and the plasm disappears. These cells, owing to their loss of protoplasm and the thinness of the walls, may become flattened and compressed by the resin in the duct. A cell of this type is represented at the left-hand side of the duct in Text-fig. 6. In yet other cells the wall remains thin and unlignified, and the plasm active for several years, usually until heartwood formation.

The lowest proportion of thin-walled unlignified cells is found in seedling and branch wood of *Larix*. Here the majority of the epithelial cells are thick-walled, lignified and devoid of plasm (Text-



Text-figs. 1-12.

fig. 4), resembling the condition in *Tsuga* and *Abies* (Text-fig. 3). In the intermediate types, represented in adult stem wood of *Larix* (Text-fig. 5), branch wood of *Picea* (Text-fig. 6), or in *Pseudotsuga*, the epithelium consists of varying proportions of thin- and thick-walled, and of living and dead cells, and is similar to that in *Cedrus* and *Keteleeria*. The highest percentage of thin-walled cells is found in the root and adult stem wood of *Picea* (Text-figs. 7-9). In such parts of this genus the majority of the cells, the thick- as well as the thin-walled, remain functionally active for many years.

An indication of the actual proportions of thin-walled cells in the epithelium in these different genera is given in Table I. This table represents a study of approximately 1000 ducts in *Pseudotsuga*,

TABLE I. Proportion of thin-walled unlignified cells in the epithelium of vertical resin ducts, expressed in percentages of the total number of epithelial cells

Species	Type of wood				
	Seedling stems	Branches	Inner wood of adult stems (at tip)	Outer wood of adult stems (at base)	Roots
<i>Larix laricina</i>	5	3	5	10	6
<i>Pseudotsuga taxifolia</i>	—	4	7	22	17
<i>Picea canadensis</i>	24	16	26	53	50

4000 in *Larix* and 5000 in *Picea*, the examination having been made from transverse sections. It may be seen that the lowest proportion is found in *Larix*, where there are fewer thin-walled cells than in such Abietinae as *Cedrus*, and is highest in *Picea*. *Pseudotsuga* occupies a position intermediate between *Larix* and *Picea*, but there is doubt if it could be distinguished with certainty from the other two genera in the case of individual specimens. It is noteworthy, however, that

Text-figs. 1-12. Fig. 1. *Pinus Strobus*, tangential section showing development of duct and segmentation of the associated cells. Fig. 2. *Abies balsamea*, radial section of duct in stem wood, showing empty epithelial cells and surrounding parenchyma cells, most of which have retained their protoplasm. Fig. 3. *Abies concolor*, transverse section showing two adjacent ducts in branch wood. Fig. 4. *Larix laricina*, transverse section of duct in seedling stem. Fig. 5. *Larix laricina*, transverse section of ducts in adult stem wood. Fig. 6. *Picea canadensis*, transverse section of ducts in branch wood. Fig. 7. *Picea canadensis*, ducts in adult stem wood. Fig. 8. *Picea canadensis*, radial section of duct in mature stem, illustrating pulling apart of epithelial cells. Fig. 9. *Picea canadensis*, transverse section of ducts in root. Fig. 10. *Pinus nigra* var. *austriaca*, transverse section of duct in axis of female cone. Fig. 11. *Pinus Strobus*, radial section of duct in stem wood. Fig. 12. *Pinus sylvestris*, transverse section of duct in stem wood.

when a large amount of material is studied similar trends become apparent in each of the three genera. The proportion of thin-walled cells is lowest in branches (either of young or old trees), seedlings, and in the inner wood of both young and old trees; and is highest in the outer wood of adult stems and in the root.

From the table it would appear that the age of the cambium is an important factor in determining the number of thick and thin-walled cells in the epithelium. The percentage of thin-walled cells is considerably less in the innermost annual rings of seedlings and at the tip of adult trees than in the outer wood at the base of adult trees.

The proximity to other ducts may be another factor modifying the structure of the epithelium. The percentage of thin-walled cells in *Picea* is slightly higher when the ducts are in tangential series and where vertical and horizontal ducts come into contact. These differences are not so conspicuous in *Larix*, though in this form there is a slight delay in lignification in such locations. The position of the duct in the ring in relation to the spring and summer wood did not appear to influence the character of the epithelium, but a different condition was found in the case of the horizontal ducts. Here there was a marked increase in the number of thin-walled cells in the summer wood.

The parenchyma cells surrounding the epithelium in these Pineae are, except for an odd instance in *Picea*, thick-walled and lignified. In *Larix*, some of these cells die during the first year, while others retain their plasm for several years. In *Picea*, where more of the epithelial cells remain alive, practically all the surrounding parenchyma cells do also.

In the stem of *Pinus* the epithelial cells are thin-walled and unlignified. Surrounding the epithelium is a row of dead parenchyma cells with thin but somewhat lignified walls. This layer has been described by Münch (1919) as air-filled. Its existence was denied by Franck (1923) but the writer has found it, as Münch (1923) reaffirms, to be a characteristic feature of the ducts in the stem. At times these cells form a complete ring, but more often the circle is bridged at one or more points by living parenchyma (Text-fig. 12). Outside the empty cells there may be living parenchyma cells again, the walls of which are usually thin, but in some cases are thickened and lignified. Cells of the latter type were observed in such forms as *Pinus edulis*, *P. Gerardiana*, *P. canariensis*, *P. ponderosa* and *P. Banksiana*. Conwentz (1890), Gothan (1909) and Bailey (1909) have described similar cells in other species.

The tissue at the ducts in the root resembles that in the stem, but in the axis of the female cone there is considerable variation. In some types of cone, where the xylem tissues are not unlike those in the stem, the epithelium and the ring of empty cells resemble those in the stem, though the outermost parenchyma cells may be more often thick-walled and lignified. In other cases, where the cone is more compact and the tissues are denser, a larger proportion of the cells have thickened lignified walls; in fact in some cases all the parenchyma cells in association with the duct are of this type. When this is true many if not all the parenchyma cells remain alive, and if disintegration of the plasm takes place it is in the epithelial rather than in the more distant cells (Text-fig. 10). Consequently there is no ring of empty cells such as found in the stem. The existence of dead cells in the epithelium, and the absence of the surrounding ring of empty cells, together with the thick and lignified character of the walls, are points of marked dissimilarity to the condition in the stem but of close resemblance to that in such other Abietineae as *Larix*, or even *Abies* and *Tsuga*.

On the character of the tissue at the resin ducts it is evident that the different Abietineae may be arranged in a series. On the one hand are *Abies* and *Tsuga*, where the cells are thick-walled and lignified, the majority dying during the year of origin. On the other hand is *Pinus*, in the stem and root of which the cells are usually thin-walled, and except for the ring of empty cells, remain active for several years. Intermediate conditions are found in the other genera. It is of interest to note that in these intermediate forms the proportion of thin-walled, living cells is lowest in the seedling and innermost wood of the branches and stems of adult trees, while outwards from the pith, with the aging of the cambium, the proportion becomes greater. Such an increasing development of thin-walled cells from the seedling to the adult stage might be considered evidence that the thick-walled character was the primitive type, and the thin-walled, as found in *Pinus*, the specialised. It might also be pointed out that in *Pinus* many of the cells at the ducts in the female cone axis, which has been considered by many anatomists to be a conservative region, are thick-walled, in contrast to the usual thin-walled condition in the stem. On the other hand, the fact must be recognised that some of the variations in the different "conservative" parts of the tree appear to be correlated with ecological or special physiological conditions and have not the phylogenetic significance that may be attributed to them. This probably applies,

for instance, to certain of the characters in the root, and also in the cone axis.

The differences in the character of the tissue associated with the ducts are paralleled by those of function. In *Abies* and *Tsuga* a comparatively small amount of resin is produced, probably owing to the early death of the cells. In *Pinus*, where the cells remain alive for several seasons, the supply is much more copious. The development of resin and the behaviour of the cells surrounding the ducts in the pine have been studied by Münch (1919). He found in *Pinus sylvestris* that the resin originates in the epithelial cells, from which it passes into the lumen of the duct. Here it collects, and as the volume increases the duct enlarges. This compresses the epithelial cells and owing to the reduction in size their osmotic potential rises. There is a tendency for water to be drawn into the epithelial cells, and when this occurs their size is increased and pressure exerted upon the resin in the duct. If the latter is ruptured, the resin is forced out. The epithelial cells may produce more resin, which once again fills the duct. The ring of empty cells surrounding the epithelium is believed to act as an air mantle, preventing too rapid entrance of water into the osmotically active epithelial cells. It is evident that this complex procedure of emptying and refilling can be best performed in ducts lined by thin-walled cells, as found in the pine. It cannot be as successfully carried out in the Abieteae, or in such Pineae as *Larix* and *Pseudotsuga*, where so many of the epithelial cells are immobile owing to their thick lignified walls and, furthermore, are dead and incapable of producing resin.

The tylosoids in the ducts of the Abietineae occur in diverse amounts and arise at different times in the various genera. In such Abieteae as *Abies* and *Tsuga* they usually occur only in those ducts in the immediate vicinity of injury. They are formed by expansion of the epithelial cells, the cells growing out into the duct before the walls thicken and lignify. The tylosoids are thus formed shortly after the origin of the duct and its surrounding tissues. In *Larix*, *Pseudotsuga* and *Picea* some of the tylosoids arise under circumstances similar to those in *Abies* and *Tsuga*. Close to wounds tylosoids may be formed during the first season, before the walls of the epithelial cells have thickened and lignified. In the ducts farther distant from the centres of injury, however, tylosoids are of more common occurrence in these Pineae than in the Abieteae. And unlike the tylosoids in the ducts near wounds, those in the more distant ducts are not as a rule formed until the time of transition

from sap- to heartwood. Since these originate by expansion of the epithelial cells that have remained thin-walled and unlignified until that time, they occur most abundantly where the proportion of such cells is highest, as for example in *Picea*, and less often in *Larix*. The walls of the tylosoids sometimes remain thin, but usually are thickened and lignified.

In *Pinus* the ducts are more completely occluded by tylosoids than in other conifers, the epithelial cells expanding into the ducts on heartwood formation, or earlier in the neighbourhood of wounds. The walls are usually lignified and thickened in varying degree in different cells. In the root the time of tylosoid formation varies greatly, and in some cases, as in the more deeply buried roots, is very much later than in the stem. This is correlated with the later heartwood formation.

The causal factor in the development of the tylosoids appears to be the entrance of air into the wood.

CHARACTER AND DISTRIBUTION OF DUCTS AT WOUNDS

The relationship between wounding and the development of ducts, particularly in such forms as the Abietinae, has been noted by many investigators. It has not been as generally recognised, however, that the character and distribution of ducts vary with the genus and the type and circumstances of injury. For this reason it is necessary when dealing with the distribution of ducts to consider the different types of wound and the different genera separately. The distribution at open-type wounds, where the cambium has been destroyed and the area healed by overgrowth from the sides, as in Pl. I, figs. 1 and 2, will be dealt with first.

At open-type wounds in *Abies* and *Tsuga* the associated ducts are almost invariably in tangential series. If the injury has been severe the series may extend several centimetres vertically and considerable distances laterally from the wound, but in the majority of cases the area of response is much less. As a rule there is only one tangential series at a wound (Pl. I, fig. 2), but occasionally a number of later series also arise, either in the same or succeeding rings (Pl. I, fig. 1). The innermost series lies a short distance outside the inner limit of wound tissue, and extends upwards and downwards with little or no radial movement in the ring. Only where the series dies out at its upper or lower ends do occasional ducts pass gradually outwards a short distance from the remainder, but the amount of such radial movement is always slight, rarely more than a few micra.

In addition to the tangential series there may be a few sporadic, scattered ducts near the centre of injury. These are always short and rarely extend as far vertically as the tangential series.

When ducts occur at open wounds in *Pseudolarix* and *Cedrus* they likewise are in tangential series. Similar tangential series may also be observed in *Keteleeria* (Pl. I, fig. 4).

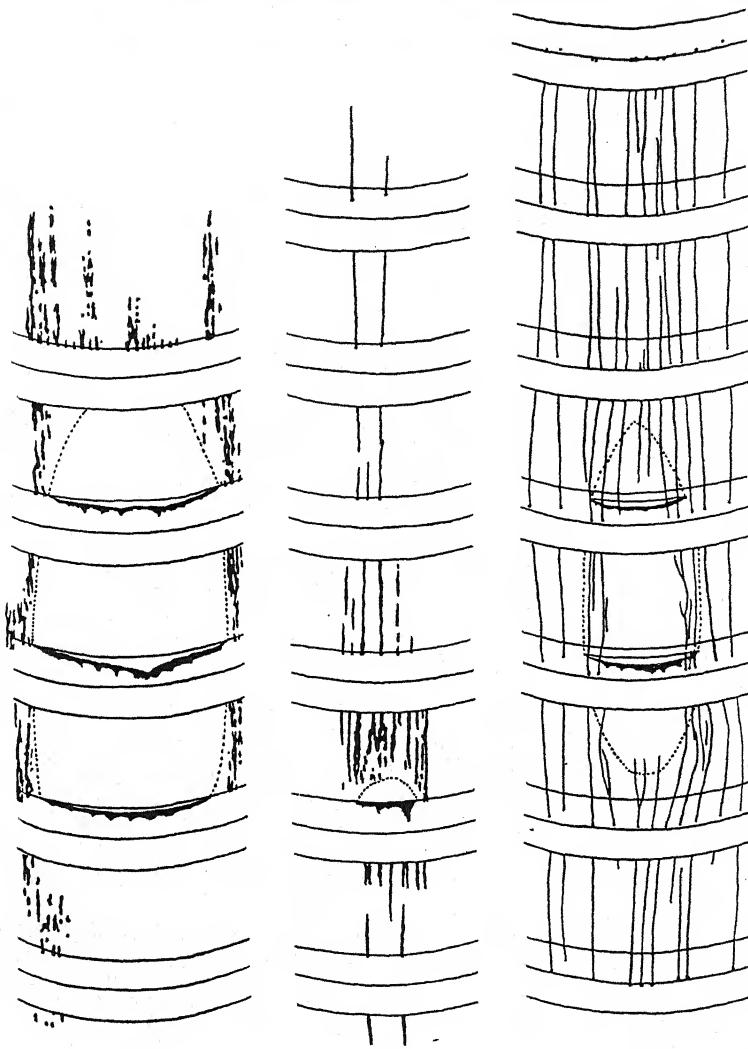
In the vicinity of open wounds in such Pineae as *Larix*, *Picea* and *Pseudotsuga* the usual condition is for the ducts to be in tangential series, as in the Abieteae. Such series are shown in Pl. I, figs. 7 and 9. In some cases, however, the ducts are farther separated and quite widely spaced. The tangential arrangement may be preserved, or the alignment may be only approximate (Pl. I, fig. 8). A few instances were observed where the ducts were so widely scattered, both radially and tangentially, that no indication of tangential alignment was recognisable.

When the ducts are in tangential series they may extend vertically above and below the wounded area with little or no radial movement in the ring, as in *Abies* and *Tsuga*. In other cases the ducts in their vertical course move both tangentially and radially in relation to one another, and since the amount of displacement varies in adjacent canals those which were in tangential series near the wound become dispersed as the distance from the injury widens; for example the two ducts above the wound in Text-fig. 14. As a result at points several centimetres above or below the wound little indication is to be seen of the original tangential arrangement. When the ducts in the immediate vicinity of the wound are not in tangential series but are scattered, these also may alter their relative positions upwards and downwards.

The distribution of ducts at open wounds in these Pineae thus varies considerably, in some cases resembling that in *Abies* and *Tsuga*, while in other cases the ducts are much more widely separated or scattered, both at the level of the wound and above and below.

The tendency toward a scattering of the ducts, noted in *Larix*, *Pseudotsuga* and *Picea*, reaches its culmination in *Pinus*. In this genus practically all the ducts are scattered, tangential series occurring only rarely.

In the few cases where tangential series do arise at open-type injuries in *Pinus*, the series are loose and the ducts widely separated. Some of the ducts in the series may follow an approximately vertical course but many become radially displaced so that at various levels



Text-figs. 13-15. Diagrams illustrating the distribution of ducts at injuries in representative Abietineae. The structures are shown as they would appear in a tangential section when viewed from above the level of the wound. The wounds are outlined by dotted lines, the ducts are represented by solid lines, and two annual rings, seen obliquely, by curved lines. Fig. 13. *Tsuga canadensis*, showing tangential series composed of short cysts. Fig. 14. *Larix laricina*, showing tangential series of cysts near wound and scattering canal-like ducts above. Fig. 15. *Pinus strobus*, illustrating the presence of true canals, some of which pass out into the succeeding ring in their vertical course, and absence of tangential series of cysts. Note: the three figures are not to be compared for the relative extent of the resin tissue since the conditions of wounding differ in each case. Magnification, figs. 13 and 14 $\times 8$, fig. 15 $\times 4$.

they pass outwards from the remainder. Accordingly such a series is never of the compact type found in the Abietinae.

At the majority of the open wounds the ducts are separate and scattered (Text-fig. 15), and only a few occur in isolated tangential groups. The ducts are further dispersed in their vertical course. Sometimes the radial movement is so pronounced that the ducts pass from one ring into the next. For instance in Text-fig. 15 some of the ducts which originated in the summer wood near the wound have passed through into the spring wood of the following year at points above and below the wound. The ducts are also displaced tangentially. Ducts which at one point in their course are separated may approach one another so that the surrounding parenchyma cells form a united mass, or the ducts themselves may fuse, in some cases only to separate again at higher or lower levels. An illustration of the amount of change in the tangential direction, and the manner in which the ducts branch and coalesce, is given in Text-fig. 16. Owing to the original irregular arrangement, and to the subsequent radial and tangential dispersal, the ducts are much more widely scattered than in the other Abietinae.

The open wound is but one of the types of injury that may be found in the conifers. It is characterised by a severe though local injury resulting in the death of the cambial cells in the affected area. In certain other types the injury is not so severe locally, but is felt over a wider area. This is true of wounds caused by the application of pressure. Along with these diverse types of wounds are found differences in the distribution of ducts.

At pressure wounds there is a tendency for the ducts to be more widely scattered than at the open wounds. In *Abies* and *Tsuga*, instead of a single tangential series, such as usually found at open injuries, there are often two or three series. A further difference may be noted in that the ducts are farther separated than in the series at open wounds. The scattering is more pronounced at pressure wounds in *Larix* and *Picea*. When the ducts are in tangential series they are often so widely separated that a dispersed distribution is approximated. In the extreme condition little or no tangential arrangement is to be observed, all the ducts being scattered, even near the centre of the injury. Finally, in *Pinus*, a tangential arrangement is seldom found. The ducts are usually widely dispersed, as in the outer annual ring in Text-fig. 21.

There are yet other types of injury, or evidences of injury, with which ducts may be associated. Thomson and Sifton (1925), in their

study of *Picea*, have shown that false rings sometimes arise as a consequence of wounding. In some cases the false rings are continuous with definite wounds, while in other cases the source of injury is not so evident but appears to be in the nature of a physiological rather than a mechanical disturbance to the cambium. The false rings vary in type, the tracheids sometimes being thicker walled than the adjoining cells, and in other instances thinner walled, as in Pl. I, fig. 6.

When ducts are found along with false rings in *Larix* and *Picea* they may be in compact tangential series (Pl. I, fig. 5), but more often are relatively widely spaced, though still in a loose and disorderly tangential arrangement, or dispersed to such an extent that tangential alignment is no longer recognisable. In the latter case the ducts do not usually follow a strictly vertical course but move both radially and tangentially in the ring, and begin and end at different levels, having no direct connection with one another. The distribution is diffuse and the definite arrangement typified by the tangential series may be quite lacking.

In the pine the ducts found along with false rings occasionally occur in loose tangential series (Text-figs. 17 and 18), but usually are more widely dispersed. The false rings shown in these figures resulted from injuries to the growing point which were so severe that development all but ceased and the axis was replaced by a lateral branch.

The distribution of ducts thus varies both with the type of wound and with the genus. When the injury affects a wider area there is a tendency for the ducts to be scattered, and at similar wounds the ducts are more widely dispersed in the Pineae than in the Abieteae.

The character of the ducts also undergoes variation. In such Abieteae as *Abies* and *Tsuga* the ducts are short and cyst-like (Text-fig. 13). In the tangential series they generally branch and fuse with one another to form a network of cavities which ramifies throughout the surrounding mass of parenchyma tissue. When compact tangential series occur in *Larix*, *Pseudotsuga* and *Picea* the ducts are cyst-like, as in the Abieteae, but when the ducts are farther separated they are more definitely canal-like. The latter type is found more abundantly distant from the centre of injury, as shown above the wound in Text-fig. 14, and in association with the diffuse types of injury, as for instance when the ducts are dispersed and there is no semblance of tangential alignment. The length of these canals varies greatly.

Near the pith, either at the base or top of the tree, the canals are comparatively short. In a small seedling of *Larix* 80 per cent. of the ducts examined were less than 0·5 cm. long, and in another

specimen 65 per cent. were under 1 cm. in length. In the inner wood at the tip of an old tree the canals were slightly longer, though still relatively short, 35 per cent. being less than 0·5 cm. long. Outwards from the centre the length increases and in the outer wood may be several times that near the pith. In an examination of the outer wood at the base of an old larch it was found that only a small percentage of the ducts were less than 10 cm. long. Mayr (1884) estimated the length in the lower half of the adult stem to be 30 cm. and in the upper half 15 cm. In *Picea* the corresponding figures were 70 and 40 cm. Evidently the length of the canals increases with the age of the wood (outwards from the pith).

In *Pinus* the ducts are generally true canals. Their length varies in different parts of the tree, but on the whole they are much longer than in the other conifers. Near the pith the maximum length appears to be in the neighbourhood of 15 or 20 cm., and the average probably 10 cm. In the outer wood Münch has found canals nearly a metre long and believes the average to be about 50 cm. A similar increase in length outwards from the pith was noted in *Larix* and *Picea*.

The lengthening of the ducts in the outer wood results in a greater extension and wider dispersal of the ducts produced subsequently to injury, and ultimately in a more uniform distribution throughout the wood. The more diffuse response in the pine as compared with the Abieteae is correlated with the greater length of the ducts in that genus.

The variations in the diameter of the ducts are without phylogenetic significance. The difference in size in a single species is shown in Text-figs. 6-9, all of which are drawn to the same scale. Similar differences have been described by Bailey and Faull (1934) in the case of the ducts in *Sequoia*.

It has been shown that the traumatic tissue in the Pineae, and particularly *Pinus*, is greatly different from that characteristic of most Abieteae. In *Abies* and *Tsuga* the response is of a definitely localised character. The ducts are short and cyst-like, they are almost always confined to tangential series which rarely extend more than a few cm. from the injury. In *Larix*, *Pseudotsuga* and *Picea* similar series may occur at wounds, but in some cases the ducts are longer and more canal-like, and may be scattered either at the wound or distant from it. The traumatic tissue is more diffuse and in many instances appears to be much more extensive than in the typical Abieteae. Finally, in *Pinus* compact tangential series never seem to occur, and looser series of ducts only rarely. The majority of the

canals are scattered and owing to their great length and subsequent dispersal are more widely distributed than in the other Abietineae. Sometimes the ducts are so widely dispersed as to simulate a normal occurrence. These generic differences in the character of the tissue resulting from injury must be taken into account when interpreting the distribution of ducts in the various Abietineae.

It is obvious that the commonly accepted differentiation between tangential series and scattered ducts as traumatic and normal is based upon an erroneous conception of the character of the traumatic tissue in the Pineae. The distribution of ducts at wounds in this group proves such a distinction between tangential series and scattered ducts to be without foundation.

A further point to be noted in connection with the distribution of ducts at wounds is that the character and amount of tissue varies not only with the type but also with the circumstances of injury. The most extensive development of ducts takes place when the injury has occurred during the growing season. The number of ducts at such wounds is augmented if there is stimulation of a chemical nature in addition to the mechanical injury. Ducts are not found at all wounds however. If the wound has been inflicted during the dormant period, as was the case in Pl. I, fig. 3, ducts are absent, or if present arise only in small numbers in the succeeding year's growth. As shown in Pl. I, figs. 2 and 3, the wounds inflicted at the different times of year may have the same general appearance but the responses are conspicuously different. The possibility of such variation must be appreciated when studying the distribution of ducts, particularly when the available material is limited in quantity. It is apparent that if such a circumstance as shown in Pl. I, fig. 3, were found in a fossil wood one would probably conclude that the wood in question did not develop ducts on injury, a conclusion which might be very much in error.

At some types of wound ducts may not occur, even though the cambium be wounded during the period of activity. Ducts are not ordinarily associated with the less pronounced frost rings, one of which is shown in Pl. I, fig. 10, nor with the bands of distorted tissue which resemble frost rings but probably owe their origin to other causes. On the other hand, as previously noted, ducts are sometimes found where there are evidences of disturbance as indicated by false rings or other abnormalities but not such definite injury as that represented by an open wound.

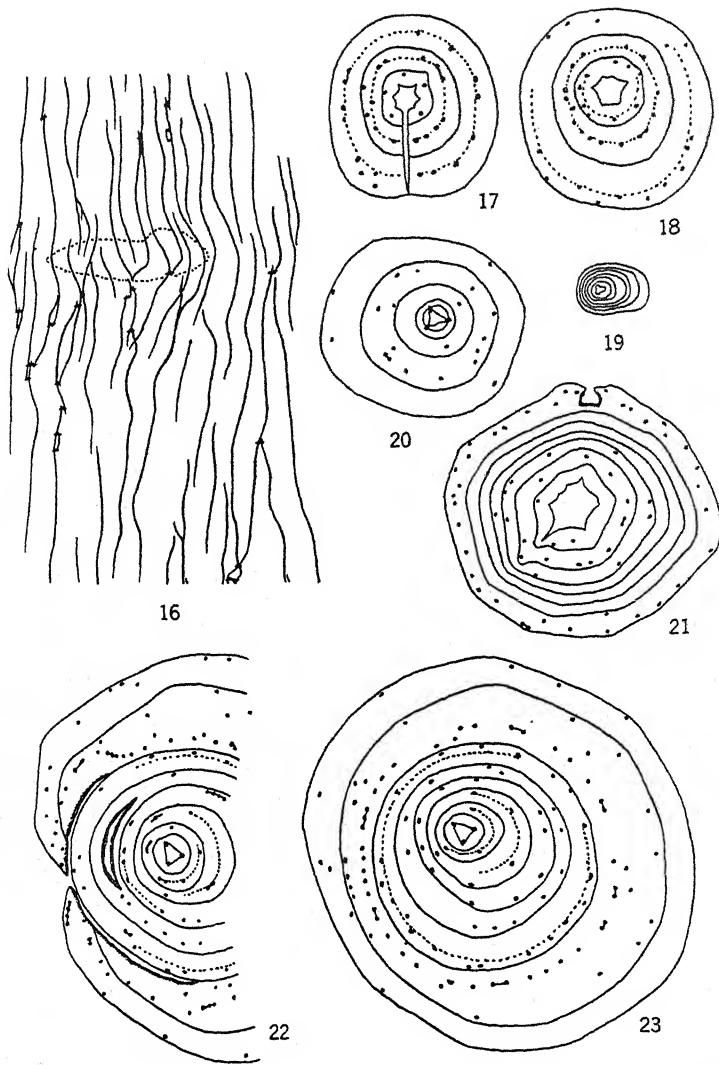
THE DISTRIBUTION OF DUCTS AND ITS INTERPRETATION

In the secondary xylem of *Abies*, *Tsuga*, *Cedrus* and *Pseudolarix* resin ducts are usually absent, except in the vicinity of wounds, where they occur in tangential series. Owing to this localised distribution the ducts have been recognised as traumatic. Certain exceptions have been reported by Jeffrey (1905) in the female cone and first annual ring of vigorous branches of one or two species of *Abies*, where ducts were observed without other evidences of wounding. Otherwise there is no doubt as to the traumatic origin of the ducts in these forms.

Tangential series are also present at injuries in *Keteleeria*, but unlike the other Abieteae, widely scattered ducts sometimes occur in addition. These appear to be of sporadic distribution. They have been observed by some investigators but not by others who have studied the conifers quite extensively (see Bailey, 1933). The relationship of the scattered ducts to injury has not been determined but probably is similar to that in the Pineae.

In *Larix*, *Pseudotsuga* and *Picea*, of the Pineae, there may be tangential series at wounds and scattered ducts in the remainder of the xylem. The tangential series have been recognised as traumatic but the scattered ducts have generally been considered to be of normal occurrence. In the case of *Picea*, however, Thomson and Sifton (1925) have shown that the ducts are not a constant feature and that they are not always uniformly distributed. They have found that ducts are usually absent from the inner growth rings of seedlings growing in protected localities, and that when ducts do occur they are aggregated in groups which are traceable to injury. In older specimens the ducts are often of more uniform distribution but even here it is considered that they are of traumatic origin. It is pointed out that the more uniform occurrence may be explained on the basis of an overlapping of the ducts resulting from different wounds. This could easily take place owing to the great length and wide dispersal of the canals and to their development at greater distances from the respective centres of injury.

In *Larix* the general distribution of ducts was found by the writer (1933b) to resemble that in *Picea*, and apparently similar conditions obtain in *Pseudotsuga*. The evidence indicates that the more widespread distribution in these genera, as compared with the Abieteae, is correlated with the more extensive and more diffuse character of the response to injury.



Text-figs. 16-23. *Pinus Strobus*. Fig. 16. Tangential view of ducts at wound, the latter represented by dotted lines. The horizontal magnification is ten times the vertical in order to illustrate the meandering course of the ducts. Fig. 17. Transverse section of seedling stem showing distribution of ducts and occurrence of false rings, these being represented by dotted lines. The section is cut at a point 6 cm. above a former bud injury. Fig. 18. Section 3 cm. above the same injury. Fig. 19. Transverse section of slowly growing seedling stem showing absence of ducts. Fig. 20. Transverse section of vigorous seedling, cut just below the junction of root and stem, illustrating irregular distribution of ducts and lack of correlation with ring width. Fig. 21. Transverse section of branch showing increased development of ducts in the outer ring, produced as a result of a ligature wound. Fig. 22. Transverse section of seedling at base, showing distribution of ducts and two wounds. Fig. 23. Transverse section cut 1 cm. above that in fig. 22. Magnification, fig. 16 $\times 4$ horizontally and $\times 0.4$ vertically, figs. 17-23 $\times 7$.

In *Pinus* ducts are more numerous and usually occur with greater regularity, but even so variations in distribution similar to those outlined in *Larix* and *Picea* may be observed. The inner rings of slowly growing seedlings, especially those found in protected locations, frequently lack ducts. Text-fig. 19 represents a transverse section of such a seedling stem. At this point there are several rings, yet ducts are absent. Where growth is more vigorous and the specimens have been subjected to a greater amount of wounding ducts appear, but their distribution is lacking in uniformity. An example is illustrated in Text-figs. 22 and 23.

In this nine-year-old specimen, ducts are much more numerous in the eighth year's growth than in the other rings. When detailed examination is made three large open-type wounds are found in this ring, one of which is shown in Text-fig. 22; the other two wounds are 15 cm. above and 6 cm. below. It is significant that the ducts are most numerous in the ring in which the severest injuries are found. Another noteworthy feature is that the ducts are not confined to the immediate vicinity of the injuries but occur abundantly throughout the length of the year's growth, and are evenly distributed around the ring. This uniformity is illustrated in Text-fig. 23, which represents a section cut 1 cm. above the wound in Text-fig. 22. Such scattering of the ducts is, as previously noted, a characteristic feature of the response in the pines.

Other wounds in this seedling are as follows: injuries to the terminal bud in the fifth and sixth years which resulted in the former axis ceasing development in each case and one of the branches becoming the stem; three open-type wounds, in addition to the three already mentioned, in the fourth, seventh and ninth years; several small wounds of various types in each year from the third to the seventh; and encircling frost rings in the eighth and ninth years. Some of these injuries, such as the frost rings, probably did not result in a development of ducts, but in other instances the ducts are clearly associated with wounds. False rings, some of which are shown in Text-figs. 22 and 23, are also connected with certain of the injuries. The distribution of the ducts is such as to leave no doubt that at least the majority are of traumatic origin. A similar distribution in relation to injury was found in the other seedlings examined.

In the stem of adult trees the ducts are often more regularly distributed than in the younger specimens. A distribution such as that noted by Münch (1919), who found a close relation between ring width and the number of ducts, however, cannot be considered proof

that the ducts are a normal feature, independent of injury. In test trees wounded for the production of resin Gerry (1931) found that the increase in number of ducts resulting from the chipping was proportional to the width of the ring, and these ducts were traumatic. The writer (1933a) has observed a similar relationship in *Tsuga*. In experimentally wounded specimens the number of ducts produced after wounding varied with the ring width, and there is no doubt as to the traumatic origin of the ducts in *Tsuga*.

Apparently there may be a correspondence between the number of ducts and the ring width when the conditions of wounding remain relatively uniform, but when the wounding is variable, such a relationship is no longer recognisable. This may be seen when the distribution in seedlings or in branch wood is studied. For instance in Text-fig. 20, which illustrates a transverse section at the base of a seedling, ducts are much more numerous in the second ring from the outside than in the larger outer ring. Similarly, in the branch illustrated in Text-fig. 21 ducts are quite numerous in the three inner rings, rare or absent in the next three, and abundant in the outer ring where a wound is to be seen. Evidently vigour of growth is a factor that modifies the distribution of ducts produced as a result of injury but in itself is not directly responsible for their development.

When considering the distribution of ducts in the pine due emphasis must be given to the character of the traumatic resin tissue in this form. In this connection it is of interest to note that Münch (1919), in his studies of trees wounded for the production of resin, found increases in the number of ducts at heights of 3 to 12 metres above the injuries at the base of the trees. This is an indication of the great distances over which the effect of injury may be felt in the pine. It is also worthy of note that the distance between the wound and the most distant of the ducts is much greater than the length of the canals themselves. Evidently ducts sometimes arise at considerable distances from the injury to which they owe their origin. Ducts of this type were observed by the writer in experimentally wounded material.

The response to injury may also be of considerable tangential extent. In wounded material a conspicuous increase in the number of ducts was often found even in that part of the ring on the side of the branch or stem opposite to the wound. This relatively uniform distribution around the ring is illustrated in the outer ring in Text-fig. 21 and in the second outer ring in Text-fig. 23. There is also considerable radial dispersal. Some of the ducts break through from

one ring to the next, so that the ducts are not necessarily confined to the year of injury.

The character of the traumatic tissue in the pines, such as the great length of the ducts, their development at considerable distances from the centre of injury, both vertically and tangentially, their meandering course effecting an even further radial and tangential dispersal, and the lack of arrangement in tangential series, results in a more extensive and widespread response than in the other Abietineae, and consequently in a more even distribution throughout the wood. The ducts are widely scattered and the localisation so evident in the Abieteae may, in some cases, be scarcely recognisable.

The degree of sensitivity to injury is a factor which also must be taken into consideration. There is evidence, both from field material and experimentally wounded specimens, that ducts arise subsequently to some types of injury in the pines when there is no parallel development in other forms.

The results of investigation show that the fairly uniform distribution which may be found in some specimens of the pine is only the expected outcome from the character of the resin tissue and the degree of sensitivity to injury. Although it may not be possible to definitely correlate each single duct with wounds, especially in field material where little is known concerning the circumstances of injury, such failure does not necessarily mean that those particular ducts are of normal occurrence and arise independently of injury. The studies of the distribution in relation to wounding show how difficult such precise correlation would be.

PHYLOGENETIC CONSIDERATIONS

Between the two subtribes of the Abietineae there appears to be no sharp line of distinction in either the character or distribution of the ducts. For instance in *Keteleeria*, considered by Jeffrey to be a member of the Abieteae, the distribution differs from that in the other members of the group by the presence of scattered ducts in addition to tangential series, and in this respect resembles such Pineae as *Larix*, *Pseudotsuga* and *Picea*. Similarly, although the epithelial cells surrounding the ducts are usually thick-walled in the Abieteae and more often thin-walled in the Pineae, there is a larger proportion of thin-walled cells in *Cedrus*, a member of the former group, than in *Larix*, belonging to the Pineae. Obviously there is overlapping between the two subtribes and for purposes of comparison the different genera may be considered as constituting a

series. Certain features of this series have been recognised by anatomists and have been phylogenetically interpreted.

Penhallow (1907) regarded the development of resin ducts as a specialisation, and considered the primitive condition to be that of parenchyma cells scattered throughout the xylem. These became zoned and aggregated, and resin cysts such as occur in *Sequoia*, *Abies* and *Tsuga* eventually arose between the cells. From cysts of this type canals such as occur in the pine were derived.

Jeffrey (1903, 1905) read the series in the opposite direction. He maintains that the general distribution of the ducts in the pine represents the ancestral condition, and that the restricted distribution in the Abietinae has been derived from that in the pine by the disappearance of ducts. As evidence in support of this hypothesis he cites the occurrence of ducts at injuries in the Abietinae, and also in the first year's growth of vigorous branches and the female cone axis of certain species of *Abies*. The argument is that the presence of ducts at wounds is a reversion, and that such parts of the tree as the first year's growth and the cone are conservative and consequently retentive of characters which otherwise have been lost. The occurrence of ducts in such locations and the absence elsewhere is believed to indicate that ducts were a characteristic feature of ancestral Abietinae.

Thomson and Sifton (1925) have reached a different conclusion from their studies of the distribution of ducts. In *Picea* they have found that ducts are absent from the seedling, or if present confined to areas of injury, while in the adult the ducts are of more universal occurrence though still traumatic in origin. This distribution is believed to indicate a phylogenetic increase of sensitiveness to wound stimuli among the conifers.

Both Thomson and Sifton's and Jeffrey's theories are founded to a large extent upon comparison of the distribution in adult wood with that in "conservative" parts of the tree, yet they arrive at opposite conclusions. Jeffrey believes the presence of ducts in wounded areas, the female cone and the first year's growth of vigorous branches to be evidence of the primitive character of the ducts. Thomson and Sifton, on the other hand, have shown that ducts are less numerous in the seedling than in mature trees. Consequently, if these different parts of the tree are all conservative in regard to resin ducts, there are obvious difficulties in reconciling the differences in distribution. The question naturally arises as to whether the conditions which bring about the development of ducts in the first year's growth or in the female cone are the same as those which

fail to produce a similar development in the adult stem. There is the possibility that the ducts in the first ring are due, not to the conservativeness of that part of the tree, but to a greater intensity of wounding, or it might be argued that the paucity of ducts in the seedling is to be explained by an absence of injury rather than a primitive lack of response.

Although the study of field material has yielded evidence indicative of a more extensive response to injury in the adult than in the seedling, the true differences cannot be fully determined from material in which the conditions of wounding are variable. For adequate comparisons the injuries must be of similar size, type and date, conditions which may be fulfilled only when the wounds are experimentally produced. This method of making similar wounds on trees of different age and on different parts of the tree was used in the following experiments.

COMPARISON OF THE RESPONSES TO WOUNDING IN THE SEEDLING AND THE ADULT

For this comparison the two native members of the Abietae, *Abies balsamea* and *Tsuga canadensis*, were chosen, owing to the restricted nature of the response to injury. Preliminary investigations were carried out on the balsam. A few small trees 0·5–1·5 m. in height and the branches of adjacent adult trees were wounded with a $\frac{1}{16}$ in. auger, the dates of wounding being June 4 and 15. At the close of the season the material was brought in and determination made of the number of ducts produced subsequently to the wounding. When comparison was made of these, little difference was found between the young and old trees after allowance was made for the differences in growth as represented by the width of the ring laid down during the year of wounding.

The experiments were repeated, this time with the more accessible *Tsuga canadensis*. The seedlings chosen for the wounding were smaller than in the case of *Abies*, the height varying from 15 to 40 cm., and the adult trees were larger. Consequently there was a greater difference in age between the smaller and larger trees. The smallest seedlings were wounded with a needle only, and the larger with both the needle and a $\frac{1}{8}$ in. half-round chisel, the needle wounds being towards the tip and the chisel wounds nearer the base. The wounds were equidistantly spaced along the axis at intervals of 8–10 cm., the number of wounds per seedling varying from two to five, dependent upon the age of the specimen. The needle wounds

were made by pressing the point into the axis until it had penetrated the wood, and in the case of the chisel wounds two contiguous cuts were made so that an ellipse of bark and cambium with a minor diameter of $\frac{1}{8}$ in. and a slightly greater major diameter was removed. The branch tips of the adult trees were wounded in the same manner. A number of branches and seedlings were wounded on June 20, and a second series on July 18.

The material was collected in the autumn and transverse sections were cut through the wound centres and at centimetre distances above and below. The numbers of ducts were determined from these sections, the greatest number noted at or near each wound being taken as the index of the response to that wound. The chief objection to this method would seem to be that the vertical extent was not

TABLE II. Average number of ducts per wound in seedlings and the branches of adult trees (*Tsuga canadensis*)

Date	Type of wound	Type of specimen	Av. ring width in mm.	Av. no. of ducts per needle wound	Av. no. of ducts per chisel wound
June 20	Needle	Seedling	0.24	5	—
		Adult	0.23	10	—
	Chisel	Seedling	0.29	—	22
		Adult	0.29	—	42
July 18	Needle	Seedling	0.22	1	—
		Adult	0.12	3	—
	Chisel	Seedling	0.27	—	1
		Adult	0.16	—	6

taken into consideration, but actually this is indicated indirectly, for the vertical extent is usually greatest where the tangential series is widest. From the figures obtained by this method the average number of cysts per wound was calculated for the branches of the adult trees and the seedlings. These averages are given in Table II, which represents a summary of 180 wounds.

In each of the four comparisons between the seedling and adult in this table it may be seen that the greatest development of ducts takes place in the adult. In the case of the woundings of June 20 the number of cysts in the branches is twice that in the seedlings, and in the wounds of July 18 the relative difference is even greater.

When comparison was made of the numbers of ducts in the different seedlings it was found that the fewest occurred in the youngest specimens, and that the number increased in the older seedlings. In some instances there were almost as many ducts in the latter as in the branches of the adult trees. This resembles the

conditions found in the investigations with *Abies*, where there was little difference between the larger seedlings and the adult.

The average amount of growth during the year of wounding has been recorded in Table II, for without due emphasis being given this factor the comparisons would be meaningless. Since the number of ducts varies with the width of the ring it is essential that the specimens compared be of similar vigour. This could not be determined with accuracy before the wounding, and on examination of the material in the laboratory it was found that some of the branches wounded on June 20 had been growing at a much slower rate than the seedlings. These were omitted from the table and only the branches and seedlings of similar growth compared. However, if the weaker branches be included, in which case the figures become: ring width, 0.20 mm.; ducts per needle wound, 9; and ducts per chisel wound, 32; there is still a greater development of ducts than in the seedlings.

The number of ducts at the wounds of July 18 is considerably less than at those of June 20. This discrepancy is probably correlated with the differences in cambial activity on the two dates. On June 20 the cambium was undergoing rapid division, but apparently activity had ceased prior to July 18. Ordinarily the cambium would still be active on the latter date, but the specimens wounded were subject to dry conditions which possibly resulted in the early stoppage of growth. They were growing on an elevation from which the land sloped sharply away on three sides, and this location, together with the scarcity of rain during the growing season, probably resulted in a low water table and a consequent curtailment of growth. This particular condition, however, has no bearing upon the differences between the seedling and adult for both were growing side by side. In any case the woundings of both dates agree in showing the same marked differences between the juvenile and adult material.

The conditions with respect to age of the cambium at the points of wounding are similar in the adult and seedling. None of the seedlings was more than 11 or 12 years old, and since the wounds were spaced along the axis from the tip downwards the age of the cambium at the wounds varied within the limits of 1-12 years. The branches of the adult trees were wounded in a similar manner and in few cases was the cambium more than 12 years old at the lowermost wounds.

The increase in the development of ducts with the age of the tree is a factor to be considered when interpreting both the differences in distribution in the adult and seedling, and the occurrence of ducts

in certain branches of adult trees, such as described by Jeffrey (1905), in contrast to the absence in the seedling. The greater sensitivity of the adult is undoubtedly a partial explanation of the occurrence of ducts in certain branches and cones on mature trees, though it seems probable that such ducts actually owe their origin to some injury or irritation of the cambium and are not simply reversions to an ancestral type as interpreted by Jeffrey. The greater development in the adult also confirms the statement of Thomson and Sifton that there is an increasing sensitiveness with age, a factor in part responsible for the more general distribution of ducts in the mature tree, though of course in making such comparisons the difference in cambial age must also be taken into consideration.

RELATION BETWEEN THE RESPONSE TO WOUNDING AND CAMBIAL AGE

Most of the experimental wounding was done in such a way that the relationship between the production of resin tissue and the age of the cambium could be determined. In each series of woundings a similar procedure was followed. The stem or branch was wounded as close to the tip as possible and at regular intervals downwards. As a result the wounds near the tip occur where there are but one or two annual rings, and those at lower levels where the age is steadily increasing. Another method, that of wounding at the same point during successive years, could be followed, but it suffers from so many disadvantages, such as the length of time required for the completion of the experiment and the complications arising from the superposition of one wound upon another, that it was not seriously considered.

The material used in the determination of the relationship between resin tissue and cambial age includes that of four species, *Abies balsamea*, *Tsuga canadensis*, *Larix laricina* and *Pinus Strobus*, wounded during the interval from 1925 to 1934. The results of 1400 of these wounds are summarised in Table III. For the preparation of this table a number of detailed ones were first constructed, in which were shown the maximum number of ducts and the age at every wound. From these figures the average number of ducts per wound was calculated for the different ages. The numbers so obtained were once again averaged for each group of five years. When this is done we find, for example, in the branches of *Abies balsamea*, wounded in 1925, an average of eleven ducts per wound between the ages 1 to 5 years and twenty-nine ducts per wound between the ages 6 to 10 years. In the other sets of wounded material the

different specimens varied considerably in the amount of growth, and since the number of ducts is modified by this factor, the material has been divided into two series: one in which the width of the wounded year's growth is more than 0·20 mm., and the other in which the width is less. The figures in Table III represent the actual number of ducts in the vicinity of the wounds in *Abies*, *Tsuga* and *Larix*; while in the case of *Pinus* only the increase over the average

TABLE III. *Relation between the development of ducts and age of the cambium*

Description of material	Average number of ducts at wounds in wood of the following age (years outwards from the pith)					
	1-5	6-10	11-15	16-20	21-25	26-30
<i>Abies balsamea</i> :						
1925, branches	11	29	—	—	—	—
1928, branches and stems, vig. < 0·20 mm.	4	8	18	—	—	—
1928, branches and stems, vig. > 0·20 mm.	12	15	22	30	—	—
<i>Tsuga canadensis</i> :						
1928, branches, chisel, vig. > 0·20 mm.	26	32	49	60	40	—
1932, branches, needle, vig. < 0·20 mm.	—	5	6	8	—	—
1932, branches, needle, vig. > 0·20 mm.	9	12	14	10	—	—
1932, branches, chisel, vig. < 0·20 mm.	—	9	13	12	23	—
1932, branches, chisel, vig. > 0·20 mm.	27	32	38	41	40	—
1933, branches, needle, vig. < 0·20 mm.	4	6	5	6	8	—
1933, branches, needle, vig. > 0·20 mm.	8	19	—	—	—	—
1933, seedlings, needle, vig. > 0·20 mm.	5	7	—	—	—	—
1934, branches, needle, vig. < 0·20 mm.	8	10	8	12	15	—
1934, branches, needle, vig. > 0·20 mm.	8	12	10	—	—	—
1934, branches, chisel, vig. < 0·20 mm.	—	20	22	27	27	—
1934, branches, chisel, vig. > 0·20 mm.	28	35	46	43	—	—
<i>Larix laricina</i> :						
1934, branches, needle, vig. < 0·20 mm.	6	8	12	13	15	—
1934, branches, needle, vig. > 0·20 mm.	13	17	—	—	—	—
1934, branches, chisel, vig. < 0·20 mm.	22	27	30	37	46	56
1934, branches, chisel, vig. > 0·20 mm.	30	45	—	—	—	—
<i>Pinus strobus</i> :						
1934, branches, needle and chisel	3	6	9	10	—	—*

* Increase in number of ducts over the average of the two preceding rings.

number in the two preceding rings is indicated. In this genus there is both an absolute and relative increase with age.

In seventeen of the twenty series listed in Table III there are fewer ducts in the first five years near the tip than towards the base. In the three remaining series no figures are given for the initial five years. Here the specimens were of slow growth, and since the wounds were equidistantly spaced, and could be made only where the diameter was sufficiently wide, wounds are either absent from the

terminal region or are too few to be necessarily indicative of the true condition. Even in these cases, however, the ducts are less numerous in the next group of five years, from five to ten, than in the older parts. After the first ten years there is a falling off in the rate of increase.

If the responses to wounds in the first five years of vigorous specimens are analysed a similar trend is found. Relatively few ducts arise from wounds at the tip in the first year, and lower in the branch, from the second to fifth years, the number steadily becomes greater.

In the table only the tangential width of the traumatic tissue is indicated, no figures being given for the vertical extent. This is usually least near the tip and increases at the wounds lower down. With the ageing of the cambium there is a distinct enlargement of the tissue in the vertical direction.

In vigorous branches of *Abies* and *Sequoia*, Jeffrey (1903, 1905) observed resin ducts in the first year's growth but not in the succeeding rings. A similar condition was not found by the writer in the experimentally wounded branches or stems of vigorous growth. The same distribution with respect to age was observed in both vigorous and weak specimens, though of course, in the former, ducts are more numerous throughout the length of the branch or stem.

The results of experimental wounding clearly show that the number of ducts increases with age when the circumstances of wounding remain uniform. A like distribution would be expected in material from the field, and actually it is found in many cases. For instance in most of the seedlings and in a large proportion of the branches of both young and old trees ducts are absent or of sparing occurrence in the inner annual rings and increase outwards. Such an absence in the first ring is illustrated in Pl. I, figs. 9 and 10. In other instances, however, there is a departure from this order of distribution and ducts are more abundant near the pith. When such is the case the evidence obtained from the experimental wounding indicates that the increase in the inner years must be the result of more intensive wounding or of special conditions which are without phylogenetic significance. The presence of wounds may be demonstrated in many instances. Often there has been breakage of the branches, damage to the bud, or abrasion and destruction of the tissues by animals. Some wounds apparently result from insect activity, and in this connection it is significant that much of the damage done by insects is near the tip in the soft, growing tissues. Furthermore, many of the injuries made by insects result in a greater

development of ducts than at purely mechanical wounds of similar size. In a few cases no definite wounds may be seen, but other abnormalities such as false rings or partial bands of thick-walled tracheids occur, which, no doubt, are indications of disturbances to the cambium of another type. In any case the results of experimental wounding demonstrate that the response to injury, as measured by the development of ducts, increases outwards from the pith, and it is this order of distribution which must be considered significant from a phylogenetic point of view.

COMPARISON OF THE RESPONSES TO WOUNDING IN DIFFERENT GENERA

The distribution of ducts in field material is indicative of a more extensive development of traumatic ducts in the Pineae than in the Abieteae, but since the conditions of wounding are variable in such material, it was considered advisable to make a test comparison of experimentally wounded specimens. In one series of experiments branches of *Abies balsamea* and *Larix laricina* were subjected to different types of pressure, such as induced by the application of pliers, clothes-pegs and wire ligatures. In another series branches of *Tsuga canadensis*, *Larix laricina* and *Pinus Strobus* were wounded with a needle and a chisel, the two types of wound being alternately spaced at 10 cm. intervals along the upper side of the branches on adult trees. The wounding was done on June 14. A total of 540 wounds was studied.

The most severe pressure injuries were those made by the pliers and the wire ligatures. In *Abies* numerous ducts were produced as a result of these injuries, the ducts being arranged in a number of tangential series extending upwards, but rarely downwards, from the wound. The ducts are very short, however, and at all but two of the wounds cease within 1 cm. Tangential series as well as scattered ducts were formed in *Larix*, and in marked contrast to *Abies*, the ducts extend above and below the wounds for distances of several centimetres. Owing to the greater length of the ducts the response in *Larix* is more extensive than that in *Abies*.

Fewer ducts were formed at the clothes-peg wounds. In *Abies* the ducts occur only in small numbers, usually in short, scattered tangential series. In *Larix* the ducts are more numerous and the tangential arrangement less distinct or sometimes entirely lacking.

When the needle and chisel wounds of *Tsuga* and *Larix* are compared it is found that the number of ducts is slightly greater in the latter. In this form the average number of ducts seen in transverse

section at or near each needle wound is 13, and at each chisel wound is 35, while in *Tsuga* the comparative figures are 10 and 27. The average width of the wounded year's growth is practically the same in both genera, 0.17 mm. in *Tsuga* and 0.18 mm. in *Larix*. Obviously this factor is not a variant. The most conspicuous difference between *Tsuga* and *Larix* is in the vertical extent of the resin tissue. In Table IV it may be seen that in the case of 39 per cent. of the needle wounds in the larch ducts occur as far as 3 cm. above and 1 cm. below the wound centre, whereas at only 2 per cent. of the needle wounds in the hemlock is the tissue of equal extent. Similar differences are to be observed in the chisel wounds. Ducts are found 3 cm. above and 1 cm. below at 64 per cent. of the chisel wounds in *Larix* but at only 10 per cent. in *Tsuga*.

TABLE IV. Comparison of the vertical extent of the response to injury in *Tsuga* and *Larix*

Species	Type of wound	Percentage of wounds where ducts may be seen at the indicated distances from the injury				
		At wound only	1 cm. above or below	1 cm. and below	3 cm. above	3 cm. and 1 cm. below
<i>Tsuga canadensis</i>	Needle	37	51	7	3	2
<i>Larix laricina</i>	Needle	18	22	20	1	39
<i>Tsuga canadensis</i>	Chisel	5	40	33	12	10
<i>Larix laricina</i>	Chisel	0	1	35	0	64

In the wounded branches of *Pinus Strobus* tangential series of the type in *Larix* and *Tsuga* were not observed, the canals being scattered. Due to this absence of compact tangential series the number of ducts in the vicinity of the wound is smaller than in the other two genera. On the other hand the ducts are more uniformly distributed around the annual ring, and in many cases there is a conspicuous increase over the number in the preceding rings even in that part of the growth on the side of the branch opposite to the wound. This more or less uniform distribution throughout the ring is illustrated in Text-fig. 23 for a seedling stem. The ducts are also very long. In the wounded branches the ducts produced subsequently to the wounding formed a continuous system extending the length of the branch. Though the actual number of ducts in the vicinity of the wound may be less in the pines than in the other Abietinae the tangential and vertical extent is very much greater. In this respect the results of the experimental wounding verify the findings obtained in the study of field material.

PHYLOGENETIC INTERPRETATIONS AND FOSSIL EVIDENCE

In the living forms it has been determined, both by examination of the distribution of ducts in field material and by comparison of the responses to similar experimental wounds, that there is a greater development of ducts as a result of injury in the adult than in the seedling. It has also been shown that outwards from the pith, both in the adult and seedling, there is an increasing development of ducts when the conditions of wounding remain uniform. Such an increase has been experimentally demonstrated in *Abies*, *Tsuga*, *Larix* and *Pinus*, representative genera characterised by differences in the type and amount of tissue traumatically induced. This enlarging of the response to injury with the ageing of the cambium and of the tree itself would, on recapitulatory principles, be considered evidence of a similar phylogenetic change. That such a sequence of change actually has taken place is indicated by the condition in the fossils.

When the fossil woods of different age are studied, certain trends of development become apparent. There appears to be no doubt that gymnosperm woods without ducts antedated those with such structures. In the earlier woods, described under the names *Dadoxylon* and *Araucarioxylon*, and found in Carboniferous, Permian and Triassic strata, ducts have not been observed.

The first fully authenticated remains in which vertical resin ducts have been discovered are the mid-Jurassic *Araucarioxylon* (*Peuce*, *Planoxylon*) *Lindlei* (Seward, 1904) and *Paracupressinoxylon cedroides* (Holden, 1913). In these fossil woods a few ducts occurred in tangential series but otherwise the wood was without ducts. Occasional tangential series have also been found in *Protopiceoxylon Wordii* (Walton, 1927), a wood of similar age. In a number of other *Protopiceoxylon* forms of somewhat later age (Upper Jurassic and Lower Cretaceous), described by Gothan (1910), Stopes (1915), Seward (1919), Edwards (1925) and Read (1932), scattered ducts occur in addition to tangential series. Bailey (1933) has shown that the general distribution resembles that in *Keteleeria*. It differs from that in *Larix*, *Pseudotsuga* and *Picea* in the usual absence of horizontal ducts. Kräusel (1916), in his list of fossil coniferous woods, believes *P. antiquius* (Gothan) to be the first-known wood of the *Piceoxylon* type. It dates from the Upper Jurassic or Lower Cretaceous.

No pine-like wood has yet been found in undoubted Jurassic rocks. This age has been attributed to *Pityoxylon eiggense*, but according to Seward (1919) there is doubt as to the horizon. In this

connection it might be recalled that *Pityoxylon chasense* and *Pinites Conwentzianus*, once believed to be Abietineous woods from the Permian and Carboniferous, are not accepted as such. The former has been proven to be a *Dadoxylon* without ducts (Thomson and Allin, 1912), while the latter was found in such a location that little credence can be given to the original determination of age as Carboniferous. With the exception of the doubtful *Pityoxylon eiggense*, pine-like woods have not been recognised in strata below the Lower Cretaceous, and even in rocks of that age are of very infrequent occurrence. Only a few forms have been found, and it is of interest to note that in some of these the distribution of ducts is unlike that generally described for *Pityoxylon*. In *Pinites Ruffordi* (Seward, 1896) many of the ducts are in tangential rows, and in *Pityoxylon Woodwardi* (Stopes, 1915) the arrangement in series is even more pronounced. Also in the Tertiary *Pinus succinifera*, Conwentz (1890) has illustrated quite an extensive tangential series. In considering the distribution in *Pityoxylon*, however, it must be recognised that some of the forms described under that name are possibly related to *Larix*, *Pseudotsuga* and *Picea*, and since tangential series occur in these living forms, their appearance in the fossils would not be unexpected. But when tangential series are found in Pityoxyla which appear to be definitely pine-like in their relationships, as *Pinus succinifera*, such distribution is of considerable significance in that it shows a greater localisation than occurs in the living pines.

A review of the fossils thus gives no support to the theory that the pine-like distribution of ducts is the primitive type. Indeed the reverse is true. The available evidence indicates that the localised and restricted distribution preceded the scattered and widely dispersed type. It also seems likely that with further discoveries and more complete investigation the largest additions will be made, not to the forms with the widely scattered ducts, but rather to those in which the ducts are localised. Ducts of the restricted type probably occurred in a much larger number of forms than at present known, but have not been found owing to their limited distribution and the relatively small amount of material available.

The palaeobotanical evidence appears to corroborate that obtained in the study of the living forms, particularly as related to the differences between the seedling and adult and the changes connected with the ageing of the cambium. This apparent parallel between ontogeny, as applied to the development of characters in the tree,

and the condition in the fossils, is suggestive of a phylogenetic enlarging of the response to injury among the Abietineae.

With respect to the primitive character of the cells associated with the ducts the evidence is not as definite as in the case of the distribution of the ducts themselves. This applies particularly to the fossil evidence. For instance the preservation may be such as to show the distribution of ducts but not sufficiently good to allow determination of the wall thickness; and no data is available as to the presence or absence of lignification. However, in the better preserved specimens the cells appear to be thick-walled when the ducts are in tangential series or when both tangential series and scattered ducts occur, and more often thin-walled when the ducts are dispersed. This parallels the condition in the living forms. A noteworthy feature of the latter is that in such intermediate forms as *Larix*, *Picea*, etc., where both thick- and thin-walled cells occur, the proportion of thick-walled lignified cells is highest in such parts of the tree as the seedling and the first year's growth, parts which have been considered conservative and retentive of ancestral characters. Although little reliance can be placed upon phylogenetic conclusions derived from the study of "conservative" regions alone, the parallel between the condition in certain of these, such as the seedling, and that in the present known fossils seems to indicate that the thick-walled cell is the primitive type.

SUMMARY

In the various genera of the Abietineae the vertical resin ducts in the secondary wood arise by schizogeny and the surrounding cells by segmentation of fusiform elements.

The type of cell associated with the ducts differs over the tree but for each genus there are characteristic limits of variation. The amount of resin produced is correlated with these differences.

In the subtribe Abietae the ducts are generally cyst-like and confined to tangential series at wounds. In the Pineae the ducts are longer, and often scattered in distribution, becoming further dispersed at a distance from the centres of injury.

The evidence obtained from the study of field material and from experimental sources indicates that the more general occurrence of ducts in the Pineae as compared with the Abietae is correlated with this lengthening and scattering of the ducts produced subsequently to injury, rather than to a normal occurrence independent of wounding as has been commonly supposed. The different genera

may be arranged in a series in which there is an enlargement and dispersion of the response to injury.

From the comparison of similar experimental wounds it has been determined that in the individual species the resin tissue resulting from injury increases from the seedling to the adult stage, and from the inner to the outer wood in both seedling and adult.

Such conditions in the living forms, together with the available fossil evidence, are indicative of a phylogenetic enlarging of the response to injury among the Abietineae.

To Prof. R. B. Thomson I am indebted for his many kindnesses during the course of the investigations.

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EXPLANATION OF PLATE I

All photomicrographs are of transverse sections of stem or branch wood, magnification $\times 30$.

Fig. 1. *Abies lasiocarpa*, showing parts of three tangential series of ducts at an open-type wound.

Fig. 2. *Tsuga canadensis*, a single tangential series of ducts at an open wound.

Fig. 3. *Tsuga canadensis*, an open-type wound without ducts.

Fig. 4. *Keteleeria Fortunei*, tangential series of ducts.

Fig. 5. *Larix laricina*, tangential series of ducts.

Fig. 6. *Larix laricina*, false ring continuous with the innermost of the two tangential series in fig. 5. The section is cut at a point 34 cm. below that in fig. 5.

Fig. 7. *Larix laricina*, tangential series of ducts 1 cm. above an open wound.

Fig. 8. *Pseudotsuga taxifolia*, showing scattered distribution of ducts at wound.

Fig. 9. *Picea canadensis*, tangential series 3 cm. above a wound. There are no scattered ducts in this case.

Fig. 10. *Picea canadensis*, frost ring in early spring wood of the second year. Ducts are lacking, both at the injury and in the vigorous first year.

Fig. 1

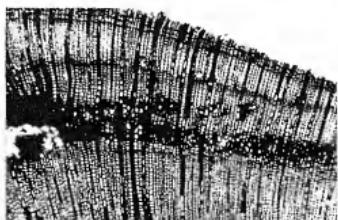


Fig. 2

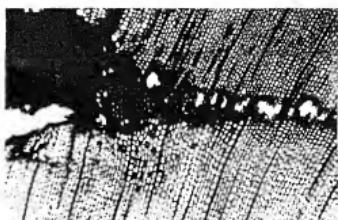


Fig. 3

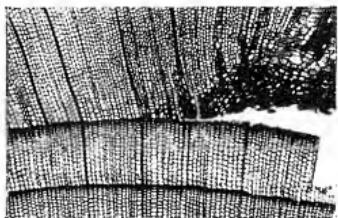


Fig. 4

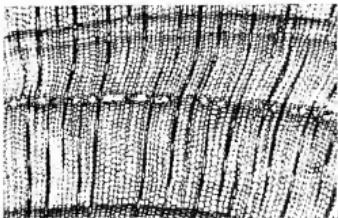


Fig. 5

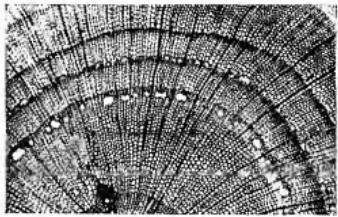


Fig. 6

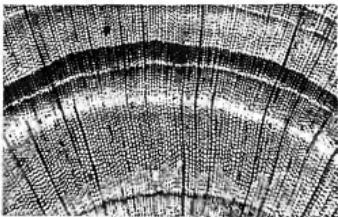


Fig. 7

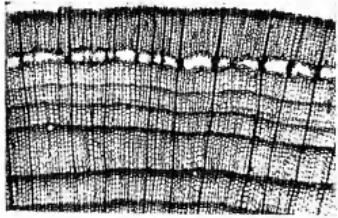


Fig. 8

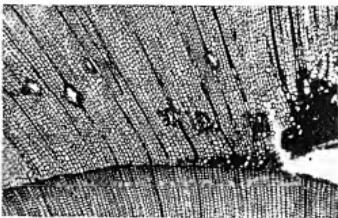


Fig. 9

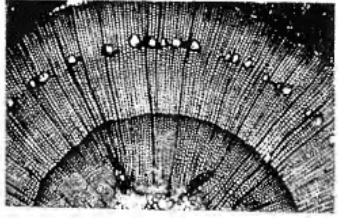
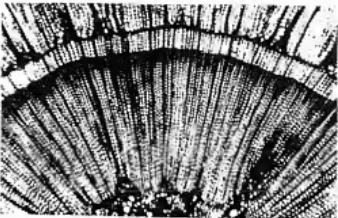
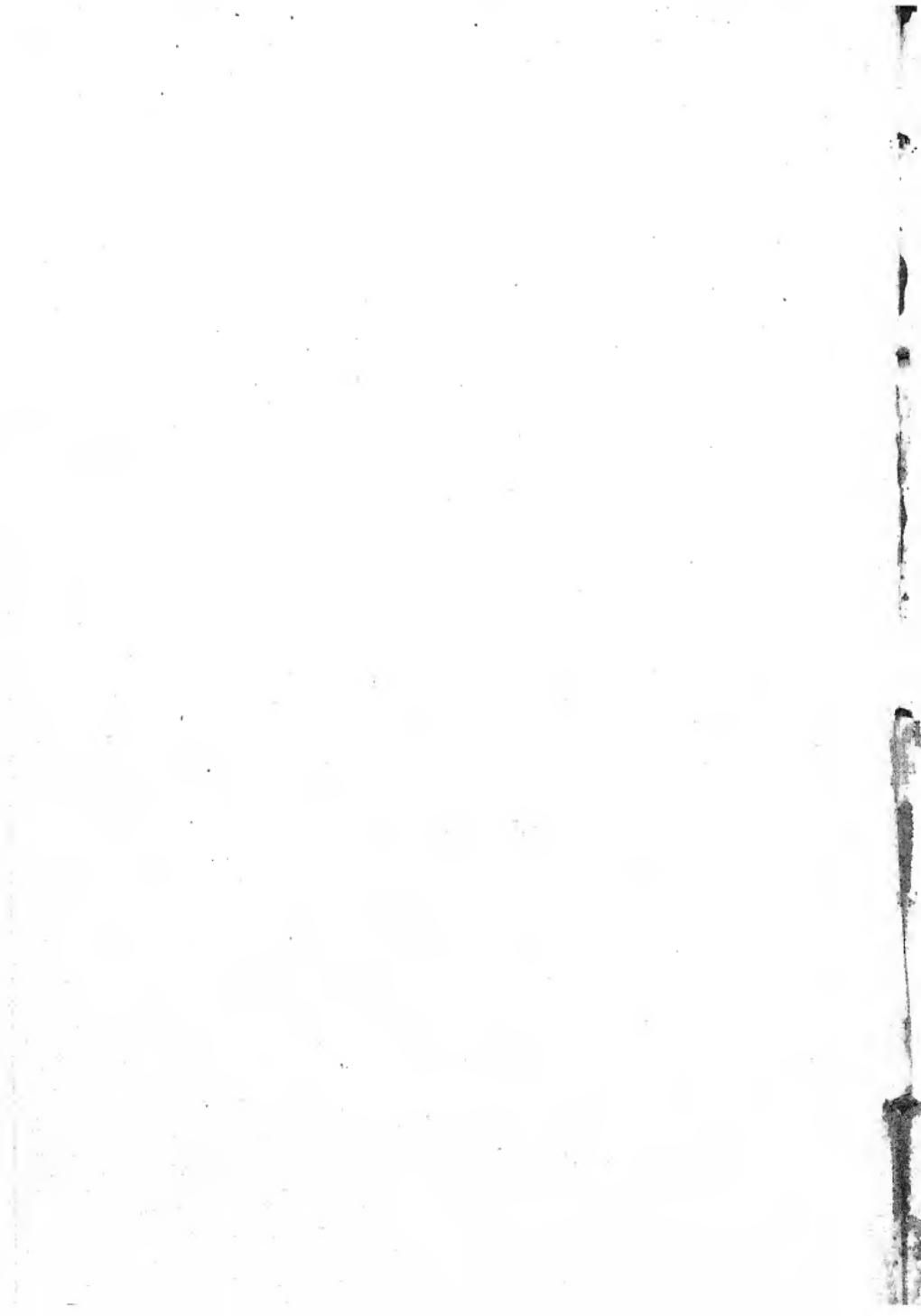


Fig. 10





THE VASCULAR GROUND-PLAN AS A GUIDE
TO THE FLORAL GROUND-PLAN: ILLUS-
TRATED FROM CISTACEAE

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(With 37 figures in the text)

THE Cistaceae afford an excellent example of a family where a study of the vascular ground-plan furnishes the key to a floral ground-plan which has always presented some difficulty.¹ To-day there is still a difference of opinion as to the number of whorls present and as to their radial relations. Widely as observers have differed in their views on these points, they have in another respect been unanimous. For each writer has in turn found himself constrained to accept the solution that in this family the law of alternating whorls must be held to break down. An examination of the vascular scheme shows, however, that such departures from the general rule as occur are not primary in their origin but arise from secondary causes. This will be evident from the following account of various forms of *Cistus*, *Helianthemum*, *Fumana* and *Halimium*.

In the most fully developed species and hybrids of *Cistus* the flower has five quincuncially arranged sepals,² all large and of about the same size, five petals, ∞ stamens and pentamerous³ carpel whorls. A few exceptional species form only three calyx segments with convolute aestivation.⁴ In the three other genera mentioned above the carpel whorls are trimerous. Where five distinct sepals are formed the two outer ones are distinctly smaller than the others; in species of *Halimium*, as in a few species of *Cistus*, they appear to be lacking altogether.

One difficulty that at once presents itself when one examines

¹ For a further consideration of this subject (now in the press) see (5).

² In the quincuncial calyx the sepals have an angular divergence of $2/5$

with their edges overlapping as shown .

³ Except in *Cistus ladaniferus*, in which the number of carpels ranges from six to ten (or even more) in each whorl.

⁴ In the convolute arrangement one edge of each sepal lies to the outside, and the other to the inside of the neighbouring sepals.

these three reduced genera is that some at least of the petals have the appearance of standing in front of the sepals. It must be mentioned that the true position of the petals in relation to the sepals is not readily ascertained by inspection owing to the inequilateral development of both sepals and petals and to the lack of prominence of the midrib which ordinarily suffices to indicate the radius of origin but which here, very generally, becomes inconspicuous or even ceases before exsertion.

Payer⁽⁴⁾ held the view that both in *Cistus* and *Helianthemum* (from which *Fumana* and *Halimium* had not then been separated) the calyx should be represented as $K\ 2+2$, but that one member of the inner whorl has become doubled, thus giving the appearance of five sepals. Also, that while in many *Cistus* species, owing to the more or less equal development of the five sepal structures, all the five petals find room for development in the intervals between them so that the two whorls regularly alternate, in *Helianthemum*, owing to the very small size of the outer sepals, this arrangement is not possible. Instead, one of the five petals is superposed upon one of the large inner sepals, and two stand in front of each of the other two inner sepals. Payer admits, however, that this disposition is very singular and that it has been an embarrassment to botanists who, nevertheless, recognise that the petals do not alternate with the sepals. The appearance seen during the early stages of development of the androecium he interprets as indicating that the numerous stamens are derived from two whorls (which, incidentally, must be assumed to be obdiplostemonous), one of five antepetalous groups, the other of five antesepalous single members.

Hofmeister⁽⁵⁾ gives a different account of the development of the ∞ stamens in *Cistus*. According to this observer the androecium is represented at first by five primordia alternating with the petals. These are shortly followed by five smaller primordia in line with the petals. Later a series of ten primordia develops beneath, and in alternation with, these earlier ten. Below, again, alternating whorls of twenty members successively make their appearance. This description, it will be noted, implies the adoption of the difficult conception that as development proceeds the number of radii upon which the ground-plan is based is doubled and then doubled again in the androecium although the number falls in the gynoecium to that of the earlier whorls.

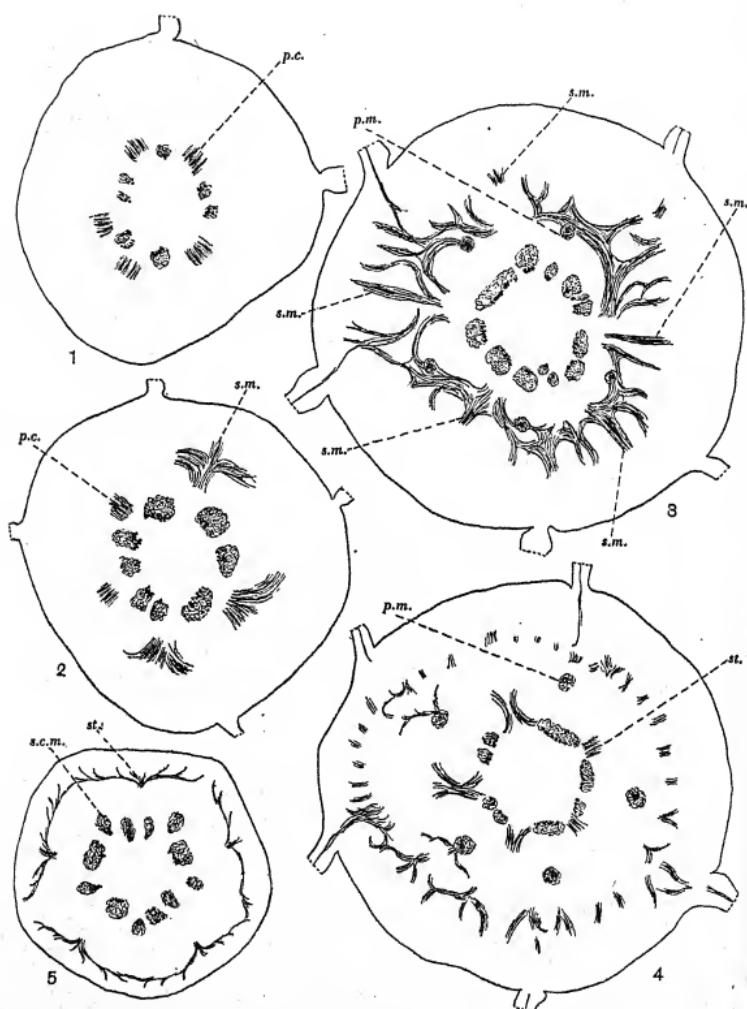
Eichler⁽¹⁾ takes yet another view, holding that neither of these interpretations is correct. Taking for examination a form then known

as *Cistus acutifolius*,¹ the only one available, it would seem, at the moment, he came to the conclusion that ten vascular bundles supplied the androecium. Of these the five outer were stronger, gave rise to a number of branches and were alternipetalous; the five inner were weaker, antepetalous and remained simple. He therefore agrees with Payer in assuming the androecium to be composed of two whorls, one of single stamens and one of groups, but takes their position to be exactly the reverse of that described by the latter observer. He attributes Payer's opposite ground-plan to an incorrect grouping of the staminal members, while Hofmeister's observation that it is the uppermost of the two first whorls of staminal members which alternates with the petals he believes is to be explained by the circumstance that as this whorl is composed of groups which require additional room these groups get pushed up to a higher level than the whorl of single stamens. In his view the flower is properly diplostemonous. The fewer stamens in *Helianthemum vulgare* Eichler holds to arise from one alternipetalous whorl which has undergone doubling (an alternate whorl of single members being presumably suppressed).

Finally yet another view has been put forward by Goebel⁽²⁾, who found himself unable to accept either Payer's conception of only two stamen whorls in *Cistus* or Eichler's of one deduplicated alternipetalous whorl in *Helianthemum vulgare*. Now this divergence of view does not arise, it is to be noted, from disagreement regarding the appearances to be observed for Goebel confirms the observations of Payer and Hofmeister as to the appearance presented by the developing androecium of *H. vulgare*, holding that an original whorl of five antepetalous primordia is followed immediately by an alternipetalous pentamerous whorl, the difference in level between the two whorls being, however, so slight that the whole ten primordia come to constitute a single whorl. In his view a succession of alternating ten-membered whorls thereafter continues regularly until the available space is completely occupied. He thus considers that there is no ground for the conception that the stamens arise in alternipetalous groups and cites in support of his interpretation the sequence observable in some Rosaceae.² It will be seen that, like

¹ To-day a form passing under the name *acutifolius* is generally referred to *hirsutus*. Whether this was the plant examined by Eichler is, however, quite uncertain, though it will no doubt have been a hybrid of some kind. This might well account for it alone being available at the time of Eichler's observations since out of the season a sterile hybrid form may be found in bloom when no flowers are to be obtained of pure species.

² As a fact, however, the vascular bundles for the numerous stamens in Rosaceae are not derived in the same way as in Cistaceae.



Figs. 1-5.

Hofmeister, Goebel envisages a plan of construction in which members of the androecium are developed upon additional radii which are not similarly utilised either previously in the development of the perianth or later in that of the gynoecium. To sum up the position: we find that there is general agreement as to the facts of external appearance and at the same time the widest divergence of opinion as to their interpretation. It will now be well to turn our attention to the vascular ground-plan which, as will appear, provides the necessary clue for the correct interpretation of the floral ground-plan in each of the above genera.

Cistus (Figs. 1-12)

The same explanatory lettering being employed for all the figures the complete list is given here to avoid repetition:

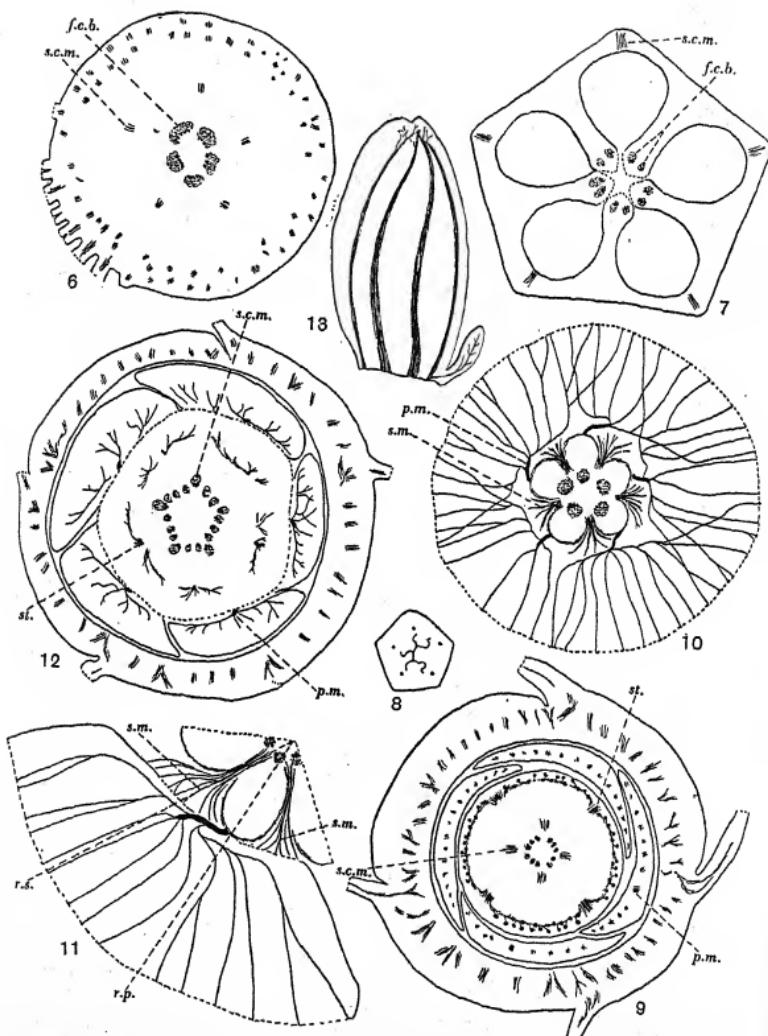
<i>f.c.b.</i>	fertile carpel bundles	<i>r.v.t.g.</i>	residual vascular tissue for the gynoecium
<i>p.c.</i>	primary cords	<i>S 1-5</i>	sepals 1-5
<i>p.l.</i>	primary laterals	<i>s.c.m.</i>	sterile carpel midrib
<i>p.m.</i>	petal midrib	<i>s.l.</i>	secondary laterals
<i>r.p.</i>	radius of petal	<i>s.m.</i>	sepal midrib
<i>r.s.</i>	radius of sepal	<i>st.</i>	stamen bundle

Though the investigations in *Cistus* were carried out mainly on hybrid forms there is no doubt that the relations described hold good in general also for the species.¹

In *C. obtusifolius*, *C. florentinus* and a white-flowered derivative of *C. incanus*, the following facts are easily made out. Five vascular cords turn out from the central cylinder at the flower base. In its

¹ Hybrid forms were employed of necessity since the observations were carried out late in autumn when flowers of any species were no longer available.

Figs. 1-5. Cistaceae. *Cistus obtusifolius*. Fig. 1. Flower base at the level at which the five primary cords which furnish the venation system of the sepals and petals turn out from the central cylinder. Fig. 2. The same at the level at which these cords begin to break up to form a central strand (sepal midrib bundle) and the horizontal tangential lateral branches which extend until they form a continuous ring. Fig. 3. The same after the five petal midrib bundles have been organised from the peripheral ring on radii alternating with those of the sepal midrib bundles. Fig. 4. The same at the level at which the five stamen bundles turn out from the central cylinder on radii alternating with those of the petal midrib bundles; two of the latter bundles have already begun to form a branch system. Fig. 5. The flower after exertion of calyx and corolla. At the periphery the five vascular systems for the five groups of alternipetalous stamens extend to form a continuous peripheral ring. Nearer the centre, on radii alternating with these systems, the five bundles for the sterile carpel midribs are already delimited. Between these bundles the residual vascular strands for the fertile carpels not yet completely organised. All from transverse sections taken at successively higher levels and magnified equally.



Figs. 6-13.

course outwards each of these cords gives off a branch on each side. These branches, which are generally much stouter than the prolonged central strand, though the latter represents the midrib bundle of the corresponding sepal, follow a horizontal tangential course in the axis parenchyma. The adjacent branches of two neighbouring sepals extend until they meet and in this way a new, outer, complete vascular ring is formed of horizontally running elements. At five points on this ring, lying in each case on a radius between two neighbouring sepal midribs a strong bundle takes its rise. This bundle becomes the midrib bundle of a petal and gives rise before exertion to a fan-shaped spread of lateral branches. The point of detachment of the petal midrib bundles is not, however, invariably strictly midway between the radii of the neighbouring sepals but may be situated somewhat nearer to the one than the other.¹

A characteristic and peculiar feature of the petal midrib bundles and one of importance in the present connection is that they do not

¹ In this connection it is worthy of note that when the bundles, which at the exertion level become the midrib bundles of the members of a whorl, arise from anastomosing strands which have turned out on the other set of radii, it appears to be not infrequently the case that alignment of these bundles on the proper radii is less strict than when such bundles spring direct from the central cylinder, or are detached from trunk cords which have previously turned out on the proper radii.

Figs. 6-13. Cistaceae (*continued*). Figs. 6-11. *Cistus obtusifolius* (*continued*). Fig. 6. The flower after exertion of calyx and corolla. Towards the periphery the numerous bundles for the ♂ stamens now distributed in a many-tiered ring. Nearer the centre the five bundles for the sterile carpel midribs. In the centre, on the alternate radii, the bundles now completely organised for the five fertile carpels. Fig. 7. The ovary at the level at which the inner face of the fertile carpels becomes defined as the pith is about to come to an end. Fig. 8. The style filament in which only the sterile carpel midrib bundles persist. Fig. 9. A thick section of a whole flower rendered transparent. The flower is exceptional in being tetramerous. Fig. 10. A thick section similarly treated, viewed from above, showing the origin on alternate radii of the vascular systems of sepals and petals, and the oblique course of the petal midrib bundles which causes the exertion points of the petals to become shifted from the true petal radii. [For the sake of simplicity the boundaries of the sepals and petals are omitted. Only the extreme basal portion of each sepal vascular system is seen, the remainder passing out of the plane of the section below.] Fig. 11. A sector of Fig. 10 more highly magnified showing the relation of the course of a petal midrib bundle to the corresponding petal radius and the neighbouring sepal radius. Fig. 12. White-flowered derivative of *Cistus incanus*. A section similar to that shown in Fig. 9 but taken at a slightly lower level. From a pentamerous flower. Fig. 13. *Helianthemum vulgare* Gaertn. One of the small outer sepals not yet disjoined from the neighbouring inner large sepal. All except Fig. 13 from transverse sections taken when in series at successively higher levels and except Figs. 11 and 13 equally magnified.

run straight to the periphery but follow an oblique course upwards and outwards. As a result the exertion radius of the petal does not coincide with the radius of origin of the midrib bundle but approaches more or less nearly to the exertion radius of the neighbouring sepal on one or other side. Herein lies the explanation of the apparent transgression of the law of alternation in the *Cistus* perianth whorls. The midrib bundles for the sepals and petals lie originally, as in the normal case, on alternating, if not strictly equidistant, radii, but the lateral twist in the course of the petal bundles and the accompanying lateral shifting of the petal exertion point cause the petals to come to stand more or less in front of the sepals. This lateral twist is due probably to a combination of conditions among which the chief may be taken to be (1) the union of the basal margins of the sepals into a continuous outer ring of tissue which for some time limits petal expansion; (2) the unequal size of the sectors of the axis occupied by the successive sepals before exertion and their inequilateral development. The direction of torsion of the corolla (which is the opposite of the calyx spiral) and the inequilateral shape of the petals are, no doubt, bound up with the oblique course of the petal midrib bundles before exertion. The above view receives support from the fact that in flowers occasionally to be met with which are tetramerous throughout not only the points of origin of the petal midrib bundles but the petals themselves stand in strict alternation with the sepals. In such flowers the four equal-sized sepals develop nearly or quite simultaneously from equal arcs of the axis circumference. The conditions are thus equal on each set of four radii, hence a typical symmetrical ground-plan results, free from the disturbance of the regular alternate relation of sepal and petal set up in the pentamerous flower. It is noteworthy, too, that in *Ascyrum*, a genus which has typically a tetramerous perianth, the relation of calyx and corolla is stated to be strictly alternate.

The alternate arrangement which, as we have seen, becomes obscured in the perianth whorls is strictly followed in the androecium and gynoecium.¹ Five bundles leave the central cylinder on radii alternating with those occupied by the petal bundles to supply the androecium. These bundles, as in the case of those destined for the sepals, give rise to lateral branches which follow a horizontal tangential course in the axis tissue until they become continuous, forming another complete outer ring. From this ring numerous strands are given off which branch freely again on their way to the periphery,

¹ Except in *Cistus ladaniferus*, see p. 47, footnote 3.

each ultimate strand entering a staminal filament. No bundles serving the androecium arise from the central cylinder on the petal radii. It is thus clear that in the above hybrid forms the androecium must be regarded as consisting of a single whorl of five alternipetalous groups. After the emergence of these alternipetalous staminal vascular bundles another set of five bundles turn outwards on the alternate (antepetalous) radii; these become the sterile carpel midribs. Hence the loculi are antepetalous.¹ The residual vascular elements left in the centre become organised into the fertile carpel bundles in line with the sepals.

It is evident from what has been stated above that in the development of the flower in these hybrids the midrib bundles for each new whorl originate in alternation with those for the preceding whorl, but that owing to a particular combination of conditions the position taken up by the fully developed petal causes this fundamental principle underlying all floral arrangement, viz. that of development on alternate sets of radii, to become obscured.

Other hybrids of the genus (*lusitanicus*, *pulverulentus*, *albidus*, "Silver Pink", and a pink derivative of *incanus*) showed a more complex vascular scheme for the androecium. In these forms a large number of separate stamen strands turn out directly from the central cylinder. These strands show no definite grouping and bear no distinct relation to either the sepal or the petal radii. They may anastomose here and there but in the main they run radially to the periphery. There is thus no definite formation of a new outer vascular ring as in the forms described above. This lack of definite arrangement suggests that the formation here of numerous strands directly from the central cylinder has come about through the shortening of the developmental process by the omission of the preliminary step in which five primary bundles leave the central cylinder before branching takes place. In this group of hybrids as in those in which these five primary bundles are present no unbranched bundle is given off from the central cylinder on the radius of each petal to pass entire into a single stamen. There can, I think, be little doubt that the outward appearance observed by Payer and Eichler in the early stages of development is not due to a whorl of single stamens alternating with a whorl of grouped stamens but is caused by the fact that after the formation of the first member in each group the second member,

¹ It is difficult to determine whether or not the loculi in Eichler's diagram of *Cistus acutifolius* are intended to be represented as ante-sepalous.

which is supplied by a lateral branch from the same vascular system, takes up a corresponding lateral position. This would explain the discrepancy in the views of Payer and Eichler. (Both of these observers, as stated above, suppose a whorl of single stamens to alternate with the groups but reverse their position.) The "candelabra" pattern of the vascular branching would also account for the illusory appearance of an androecium composed of successive whorls in which the numbers were doubled.

The above interpretation of the *Cistus* androecium receives confirmation when comparison is made with some forms of *Helianthemum vulgare* (see Fig. 25) and *H. lunulatum*. In some cultivated strains of these species the androecium vascular scheme differs from that of *Cistus* only in the feature that the spreading branches of the five primary bundles do not become continuous. The five original systems remain separate, thus affording conclusive proof that the whole number of stamens is made up of five alternipetalous groups.

We have now to consider briefly the small group of species (*C. ladaniferus* L., *C. laurifolius* L., *C. Clusii* Dunal, *C. Bourgaeanus* Coss., *C. sericeus* Munby) in which the calyx consists of only three segments of about equal size. This appearance has been taken to indicate that here the two outer sepals, present in the rest of the genus, are missing. In view of the unusual mode of origin of the petal midrib bundles in the species with five distinct sepals it was to be expected that the disappearance of these two members would disturb the symmetry of the vascular scheme and involve some definite rearrangement. When these exceptional types are examined microscopically it becomes clear, however, that the vascular scheme has not undergone modification, that the tissues of all five sepals are, in reality, present, and that what has happened is that the process of segmentation has failed to the extent that sepal 1 remains completely fused with sepal 4 and sepal 2 similarly with sepal 5. For here, as in the species with five distinct sepals, five primary cords turn outwards at the flower base. These cords behave in the manner described above. Lateral branches form a peripheral ring from which the petal midrib bundles are detached on the intervening radii, while strands which continue outwards from these cords together with others which spring from the peripheral ring serve the calyx. When the three segments become disjoined segmentation occurs at such points that one segment receives the strands arising from one of the primary cords and from that arc of the peripheral ring extending between the points of origin of the petal midrib bundles to right and

left, while each of the other two segments receives the strands derived from two of the five primary cords and from two similar arcs of the peripheral ring. It is thus evident that of the three segments one alone corresponds to a single sepal and that each of the other two represents two conjoined sepals. Confirmation of this interpretation is afforded by the other three genera cited above which together furnish a series of grades ranging from complete segmentation into five sepals of which, however, two are much reduced in size, through stages of imperfect segmentation, to that in which sepals 1 and 2 remain completely and permanently conjoined with sepals 4 and 5, respectively. It remains to consider these genera in rather more detail.

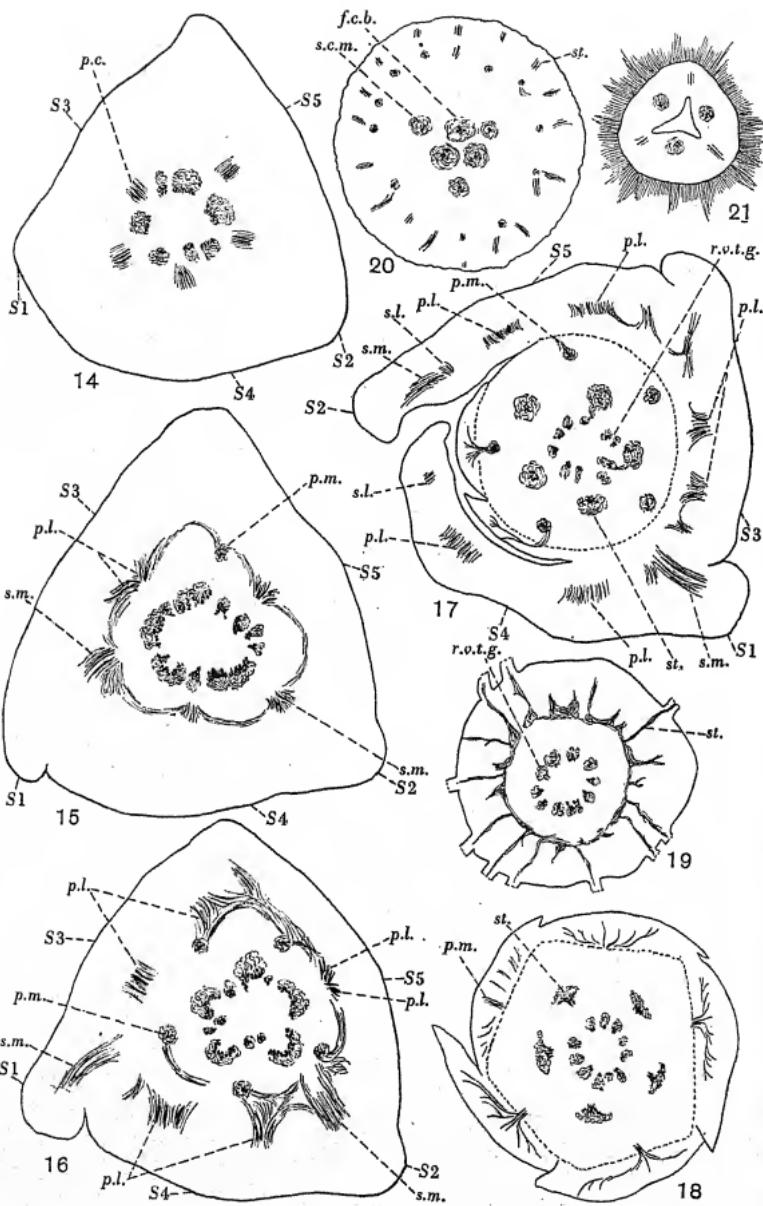
HELIANTHEMUM, FUMANA, HALIMIUM (Figs. 13-33)

In all three types the mode of origin and relations of the vascular bundles for corolla and androecium are similar to those described for *Cistus obtusifolius* except for the less extensive development in some forms mentioned above (p. 56). We can therefore confine our attention to the calyx and gynoecium. In the gynoecium, owing to the disappearance of two carpels in each whorl, both whorls in all three genera are not only apparently, but genuinely, trimerous.

HELIANTHEMUM VULGARE GAERTN. (Figs. 13-23 and 33)

A transverse section of the flower base has a triangular outline, the small outer sepals 1 and 2 being situated at two of the angles and the large inner sepals 3, 4 and 5 occupying the three sides. As the exsertion level is reached and the sepals begin to separate, sepal 1 becomes free at its one edge from sepal 3 but remains for a short time conjoined by its other edge with sepal 4. Similarly, sepal 2 becomes free on the side adjacent to sepal 4 but remains fused at first by its other edge with sepal 5, becoming entirely free, however, as a rule, before sepal 1. In the meantime the contiguous edges of sepals 3 and 5 (which constitute the third angle of the section) have also separated, exhibiting a considerable overlap. These two sepals are overlapped on their other edge by the small outer sepals 1 and 2, respectively, but neither overlaps nor is overlapped at this level by sepal 4, each extending only just so far as to come into contact with this latter member. These relations are shown in Fig. 33.

The development of the vascular system of the calyx follows in the main the same course as in *Cistus*. Typically five primary cords leave the central cylinder approximately in line with the two angles



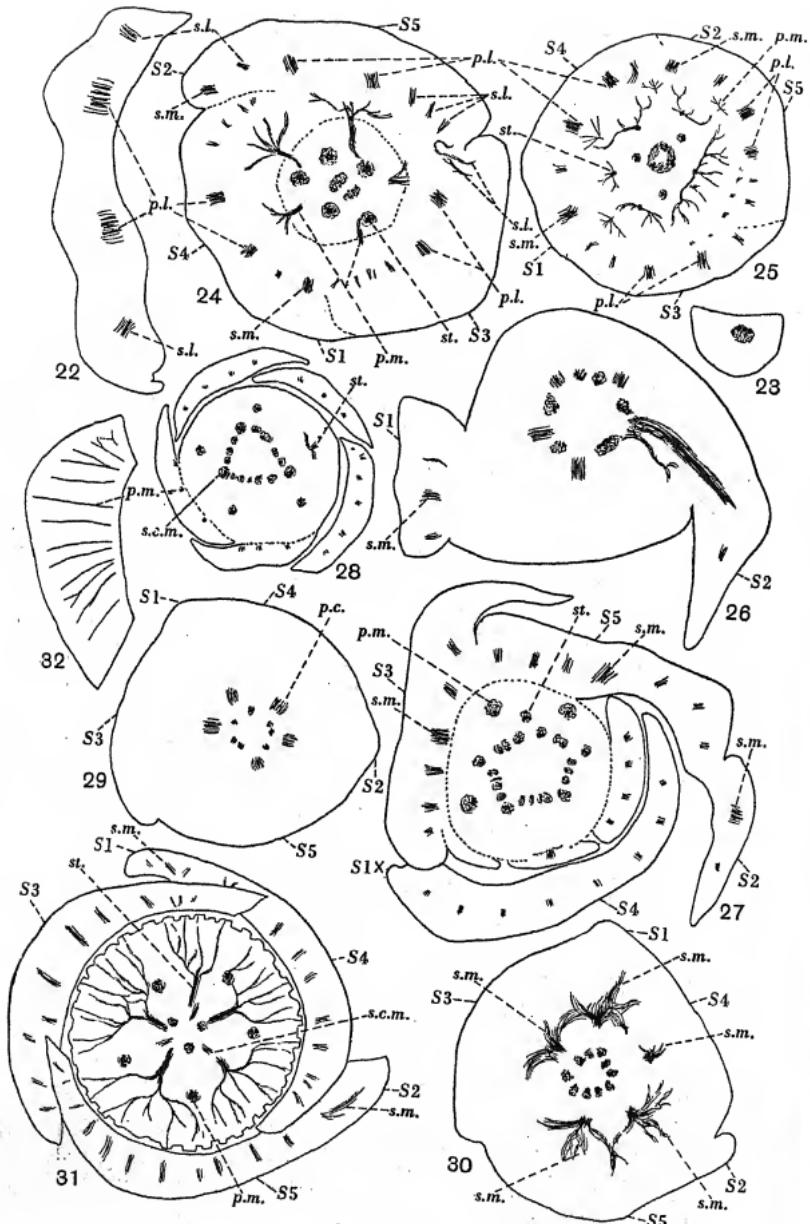
Figs. 14-21.

and three sides occupied by the sepals and give rise by anastomosis of the first formed lateral branches to a peripheral ring from which the petal midrib bundles are detached on intervening radii. From the two cords in line with the angles a strand is prolonged which enters the corresponding sepal, usually without further branching, and becomes the midrib. The cords in line with the three large inner sepals, on the other hand, develop no central strand beyond (i.e. outside) the peripheral ring, for these sepals are without a midrib. They show a characteristic four-veined pattern, a pair of strong laterals near the centre and a weaker pair near the margins. The secondary (marginal) laterals may spring either from the primary (central) laterals or from the peripheral ring in the case of both halves of sepal 3 and of the one half of sepals 4 and 5; but in the other half of these two latter sepals they are usually carried out for some distance conjoined with the adjacent midrib bundles of sepals 1 and 2, respectively.

In their mode of origin and in their oblique course below the exsertion level the petal midrib bundles resemble those of *Cistus*. Also, as in *Cistus*, the exsertion radii of the petals alternate with those of the sepals but the divergence from a strict intermediate position is more marked. As Payer has suggested, the great difference in the size of the sectors occupied by the large and small sepals, respectively, renders a strict relation between sepals and petals out of the question,

Figs. 14-21. Cistaceae (*continued*). *Helianthemum vulgare* Gaertn. (*continued*).

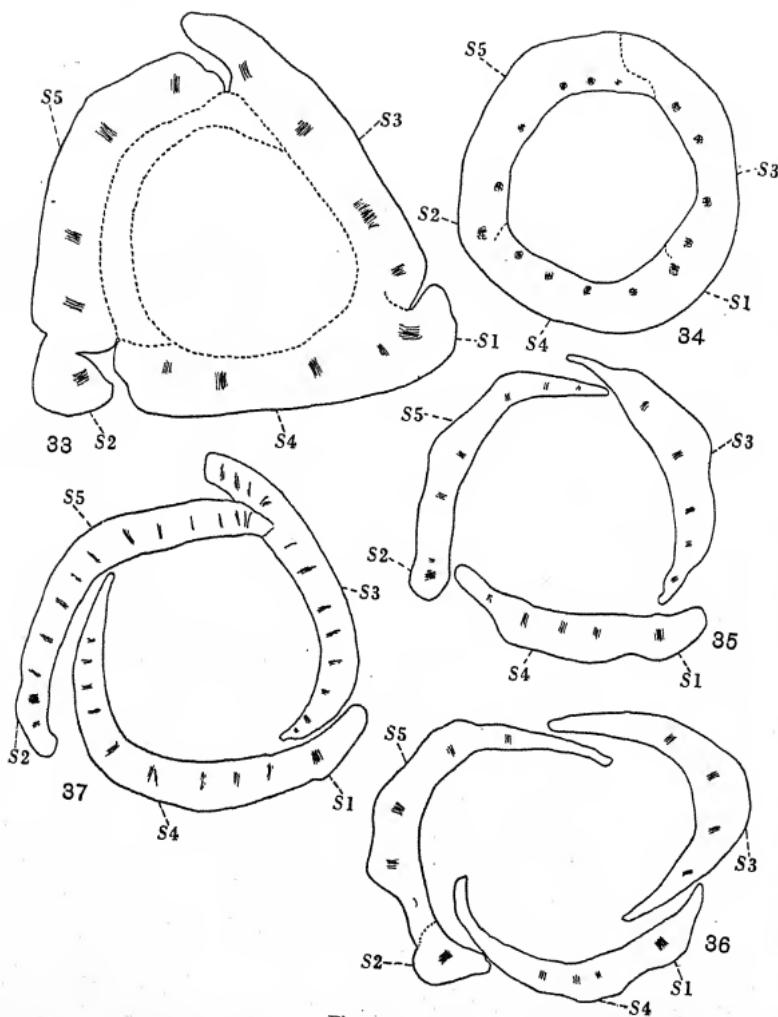
Fig. 14. Flower base. The five primary cords which supply the sepal and petal systems have turned outwards from the central cylinder. Fig. 15. The same after the primary cords seen in Fig. 1 have given rise to branches which form a continuous peripheral ring from which the midrib bundles for sepals 1 and 2 and the branch systems for sepals 3, 4 and 5 are becoming delimited. Fig. 16. The same after the petal midrib bundles have been delimited from the peripheral ring. Fig. 17. The same after partial exsertion of the calyx. In this specimen, disjunction of the sepals is completed first between sepals 2 and 4, consequently the petal bundles are further developed on this than on the opposite side of the flower. Nearer the centre on the alternate radii the five stamen bundles. In the centre the residual vascular strands which serve the gynoecium. Fig. 18. The flower after exsertion of the calyx. At the periphery the five petals. Nearer the centre the vascular strands for androecium and gynoecium as in Fig. 17. Fig. 19. The same after exsertion of the corolla showing the peripheral ring formed by horizontal tangential branching of the five stamen bundles. Fig. 20. The same after the residual vascular elements for the gynoecium seen in Fig. 19 have become organised into the bundles for the three sterile and three fertile carpels. At the periphery the bundles for the stamens. Fig. 21. The ovary after the appearance of the loculus. [This figure should be turned through 180° in order to make the orientation accord with that of Fig. 20.] All from transverse sections taken at successively higher levels and magnified equally.



Figs. 22-32.

for the petals are of uniform size. If, therefore, equal spacing of the five equal-sized petals is to be maintained, the radial relation of individual petals to sepals will not be identical. But equal spacing of the equal-sized members of a whorl is of the essence of the developmental rhythm. The alternation in the above circumstances can only approach as near to exactitude as this fundamental rhythm will permit. But the precise radial position of the *exsertion points* is not, for the reasons set out above, of any significance. The point of importance is that there is no departure from the general rule as regards alternation of the radii on which the sepals stand and those

Figs. 22–32. Cistaceae (*continued*). Figs. 22, 23. *Helianthemum vulgare* Gaertn. (*continued*). Fig. 22. An inner sepal immediately above the exertion level showing two strong primary laterals near the centre and two weaker laterals near the margins. Fig. 23. An outer sepal at the same level showing the unbranched midrib. Fig. 24. *Helianthemum lunulatum* DC. Flower base below the exertion level of the calyx. The small outer sepals, S_1 , S_2 , the position of which is indicated by the midrib bundles (*s.m.*) are still conjoined with sepals 4 and 5 respectively. The break in the outline above on the right is caused by the separation of the edges of sepals 3 and 5. The midrib bundle of sepals 3, 4 and 5 has been replaced by laterals. Within the calyx ring the vascular systems of the five petals in different stages of development. Nearer the centre the bundles for the five stamen groups are already defined. In the centre the residual vascular elements for the gynoecium (exceptional in this specimen, the carpel whorls being tetramerous). Fig. 25. The same from a form passing under the name *H. ovalifolium*. The calyx bundles and sepal boundaries as in Fig. 24 but the sepal edges have not yet begun to separate. Within the ring of calyx bundles the bundle systems of the five petals. Nearer the centre the five bundle systems (in this specimen very unequally developed) for the androecium. Still nearer the centre the bundles for the three sterile carpels and a ring from which the bundles for the three fertile carpels are organised. Figs. 26–28. *Fumana Spachii* Gren. and Godr. Fig. 26. The same shortly before exertion of sepal 1. Fig. 27. The same after exertion of sepal 1 (position indicated by \times) and partial exertion of the others. Sepal 2, though otherwise free, is still conjoined with sepal 5. The petals are also seen in process of exertion. The five bundles for the androecium are turning out from the central cylinder on the alternate radii. Fig. 28. The flower after exertion of the calyx. The bundles for the three sterile carpels can now be identified in the residual vascular ring. Figs. 29–32. *Halimium ocyoides* Willk. Fig. 29. Flower base at the level of origin of the five primary cords which turn out opposite two angles and three sides of the triangular outline. Fig. 30. The same after the cords seen in Fig. 29 have begun to anastomose. Fig. 31. The flower at the level of exertion of the calyx showing that one of the three structures consists of a single sepal and each of the others of two conjoined sepals. Near the centre the five bundle systems for the androecium alternating with the five petal midrib bundles. [For the sake of simplicity the branch systems formed by the petal bundles are omitted.] In the centre the bundles for the three sterile carpels and on the alternate radii those for the three fertile carpels. Fig. 32. The basal region of a petal showing characteristic asymmetry and inconspicuous midrib. All except Fig. 32 from transverse sections taken, when in series, at successively higher levels and equally magnified.



Figs. 33-37.

on which the petal midribs take their rise. The order of development of the five petals appears to vary according to the order in which the sepal edges separate. Thus in Fig. 17 the most developed petals are adjacent to the free margins of sepals 2 and 4, whereas when sepals 3 and 5 separate from one another before sepal 2 is wholly disjoined as in Fig. 33 the adjacent petals on either side of this break develop first.

A slight variation from the above general scheme was observed in some specimens. In these exceptional flowers six cords leave the central cylinder at the flower base to supply the perianth. The additional sixth cord arises a little later than the normal five and turns out on the radius of junction of the two large sepals which are not separated by an intervening small sepal. It then breaks up and thus provides the marginal veins in the adjacent halves of these two sepals (3 and 5) which in individuals developing five primary cords spring, like the other corresponding laterals, from the peripheral ring.

A symmetrical ground-plan having been established in the corolla strict alternation becomes possible in the following whorl and the five primary androecium bundles alternate in regular manner with the petal midrib bundles. This relation is particularly clearly seen in a strain, apparently of garden origin, passing under the name of *Helianthemum ovalifolium* (Fig. 25) and also in *H. lunulatum* DC. (Fig. 24). For in these smaller-flowered types the floral vascular system is less extensive and the plan therefore more obvious. There is no formation at the periphery of a continuous ring through

Figs. 33-37. Cistaceae (*continued*). The calyx of some forms of *Helianthemum* and *Halimium* showing the stages by which sepals 1 and 2, though still present, cease to assume independent form. Fig. 33. From a flower of *Helianthemum vulgare* just before exertion. Sepals 1 and 2, both of which at a higher level become completely disjoined from their neighbours, are here still conjoined with sepals 4 and 5, respectively. [The outer interrupted boundary line indicates the inner limit of the calyx tissue, the inner one that of the corolla tissue which, at this level, is only defined on the left.] Figs. 34, 35. From a bud of a form passing under the name of *H. ovalifolium*. Fig. 34. Immediately after exertion. All five sepals are still united, forming an unbroken ring. Fig. 35. Immediately after the break-up of the ring seen in Fig. 34 into three segments. Sepals 1 and 2 are still conjoined at this level with sepals 4 and 5, respectively. Their presence, indicated in the outline of the corresponding segments, is confirmed by the presence of their midrib bundles. Both become completely disjoined at a higher level. Fig. 36. From another bud of the same strain immediately before complete disjunction of sepal 2 from sepal 5. Sepal 1 becomes free from sepal 4 a little later. Fig. 37. *Halimium ocyoides*. The three segments after exertion and just before they separate. The contours of sepals 1 and 2 are less marked than in *Helianthemum ovalifolium* and neither attains independent form but the corresponding midrib bundles are present as in this latter type.

anastomosis of the sepal branch systems. As a result the petal bundles do not originate peripherally but are differentiated in the more usual way from the central residual elements so that their course is directly outwards from the first. The five primary stamen bundles which lie on the alternate radii also give rise to less extended branch systems which remain separate or, at least, form no continuous ring and hence afford the clearest proof that the whole stamen ring is made up of five alternipetalous groups.

FUMANA SPACHII GREN. AND GODR. (Figs. 26-28)

From the present point of view the chief interest of this species lies in the fact that as regards certain features of the calyx it occupies an intermediate position between *Helianthemum* and the general *Cistus* type. The two outer sepals are less reduced in size and all five have a better developed vascular system than in *Helianthemum*, the three larger as well as the two outer, smaller ones having a distinct midrib bundle. As a result, possibly, of its slightly larger size, sepal 1 does not, as in the latter genus, remain conjoined at exertion with sepal 4 but at once becomes wholly free. Sepal 2, however, behaves as in *Helianthemum*, only becoming disjoined from sepal 5 after exertion. The mode of origin of the vascular bundles for the corolla and androecium and the relations of these whorls to each other and to the calyx are the same as in *Helianthemum*.

HALIMIUM OCYMOIDES WILLK. (Figs. 29-32 and 37)

This species in contrast with *Fumana Spachii* stands lower in the scale than *Helianthemum* as regards segmentation of the calyx (compare Figs. 29-31 with 26, 27 and 33-36), only three entire segments being formed. An examination of the vascular scheme shows, however, that five primary vascular cords turn outwards at the flower base as in *Helianthemum* and *Fumana* but the two bundles which in these latter genera provide the midribs of sepals 1 and 2 here, as in the few species of *Cistus* with three calyx segments, run in the adjacent marginal region of sepals 4 and 5, respectively. It is thus evident that the process of sepal union begun in *Fumana* and carried further in *Helianthemum* is here complete, and that two of the segments do not represent single sepals but two sepals fused along their margins, hence we should properly regard the calyx throughout the Cistaceae as pentamerous.¹ Here, it may be noted,

¹ In accord with other cases of fusion such as occur e.g. in the keeled segment of a *Cypripedium* calyx and a papilionaceous corolla.

we have a fresh example of the persistence of vascular systems when the members which they serve no longer take separate shape. Since the reduction to three segments does not result from the total disappearance of two members of the whorl but from a failure in segmentation, such non-disjunction will not affect the disposition of the petals, which occupy the same positions as in the types with five distinct sepals, as also do the five stamen groups.

It remains to clear up certain points in regard to the gynoecium of those types having trimerous carpel whorls. It is stated in some systematic works that the carpels (i.e. on the present view the *sterile* carpels) in *Fumana* alternate with the large sepals, while in *Helianthemum* and *Halimium* they are superposed upon these members, and this supposed difference has been used as a basis for classification. Such a reversal of the relations in types having an identical ground-plan in the intervening whorls (corolla and androecium) is noted by Eichler as curious, but a detailed examination shows that in point of fact no such distinction can be drawn between these genera, as is evident from the appearance seen in Fig. 31.¹ In all, alike, one of the three sterile carpel midribs turns outwards midway between two stamen bundles, while the other two are neither strictly alternate, nor strictly in line, with the adjacent stamen bundles, a natural outcome of equal spacing when a trimerous whorl follows successive pentamerous whorls. It may be added that throughout the family, owing to torsion, the true relations of the whorls can only be appreciated by regarding the position of any one whorl simply in relation to that of its immediate predecessor.

SUMMARY AND CONCLUSIONS

A study of the vascular ground-plan in the flower of Cistaceae leads to the following conclusions:

1. That the apparent failure of the principle of alternation in the relation to one another of successive whorls in this family is not fundamental but is of secondary origin.
2. That the midrib bundles for sepals and petals arise, as normally, on alternating radii, but that owing to the peculiar mode of origin of the latter bundles from a peripheral ring of secondary origin this alternation is less exact than in the ordinary case where they, like the sepal midribs, turn out from the central cylinder.

¹ It should be noted that the position of the sterile carpel midribs in the specimen represented in Fig. 25 is probably not typical but is to some extent affected by the very unequal degree of branching of the five stamen bundles.

3. That as a result of this mode of formation of the petal midrib bundles their point of origin is not definitely fixed but may range slightly to one or other side of the proper radius.

4. That another unusual feature characteristic of this family, viz. the oblique course followed by the petal midrib bundles in their passage through the parenchyma of the axis, brings about a shift of the exsertion points of the petals still farther away from the true petal radii.

5. That this torsion of the petal midrib bundles is probably the result of compression exerted by the encircling calyx tissue, in which result the different exsertion width of individual sepals, their inequilateral development and quincuncial arrangement, possibly, all play a part.

6. That in certain hybrid forms of *Cistus* and in *Helianthemum*, *Fumana* and *Halimium* the continuous ring of stamens seen in the fully developed flower is composed of five alternipetalous groups, no development of single stamens taking place on the petal radii as supposed by some observers.

7. That in other hybrid forms of *Cistus* in which the stamens do not appear to originate in five bundles this absence of obvious grouping is probably due to the very early stage at which branching of the vascular strands which serve the androecium takes place.

8. That in *Cistus*, in which the gynoecium is, with one exception, isomerous with the preceding whorls, the sterile carpels and loculi arise in strict alternation with the bundles of stamens.

9. That in *Helianthemum*, *Fumana* and *Halimium*, in which the carpel whorls are trimerous, one sterile carpel (and loculus), generally the one more or less in line with the overlap of sepals 3 and 5, alternates strictly with the neighbouring stamen bundles while the other two, since all three are equally spaced, are neither directly in line, nor strictly alternate, with the other stamen bundles. It follows that the statement made in some systematic works, that the (sterile) carpels alternate with the inner sepals in *Fumana* and are superposed upon them in *Helianthemum* and *Halimium* and that this difference in position has classification value, cannot be maintained.

10. That the genera *Cistus*, *Fumana*, *Helianthemum* and *Halimium* form a series in which the calyx shows progressive stages in reduction as follows:

Cistus spp. All five sepals large, approximately equal in size and exserted separately.

Other spp. The two outer sepals distinctly smaller than the other three. All exserted separately.

Other spp. Calyx of three convolute segments. All five sepals are present but sepals 1 and 2 are completely and permanently fused with sepals 4 and 5, respectively, hence only one of the three segments corresponds to an individual sepal.

Fumana spp. Two outer sepals (1 and 2) considerably smaller than the three inner sepals. Sepal 1 exserted separately, sepal 2 exserted conjoined with sepal 5, becoming wholly free later.

Helianthemum, *Halimium* spp. Sepals 1 and 2 reduced still further in size. In *Helianthemum* spp. sepal 1 exserted conjoined with sepal 4, and sepal 2 with sepal 5, both becoming wholly free later.

Halimium, other spp. Calyx of three convolute segments made up of five sepals as in the corresponding forms in *Cistus*.

The species of *Cistus* and *Halimium* with only three calyx segments afford examples of the persistence of vascular bundles when the members to which they belong no longer attain separate form.

ii. The two small outer structures of the flower in both *Lechea* and *Hudsonia* were found to exhibit the same relations as sepals 1 and 2 of *Helianthemum*, but in *H. ericoides* the free portion forms only a short tooth.

I wish to express my grateful thanks to Miss D. F. M. Pertz, to whom I am greatly indebted for the accompanying figures. I also wish to thank the Director of the Cambridge Botanic Garden for material.

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DEVELOPMENT OF SPORES AND GAMETO-
PHYTES IN CERTAIN NEW WORLD
SPECIES OF *SALVIA*

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(With 50 figures in the text)

THE species investigated comprised both native and introduced forms found growing in the vicinity of Redlands, California. Native species were the black sage, *Salvia mellifera* Greene; the white sage, *S. apiana* Jepson; and chia, *S. columbariae* Benth. The introduced forms were the scarlet sage, *S. splendens* Ker, introduced from Brazil; *S. leucantha* Cav., introduced from Mexico; and *S. greggii* Gray, also introduced from Mexico.

The nomenclature of the three wild species enumerated above is that of Jepson (1923) and Munz (1927). According to Engler and Prantl (1897), *S. mellifera* Greene is placed in the genus *Ramona* and there given the name *R. stachyoides* Briquet. By the same authorities *Salvia apiana* is given the name of *R. polystachya* Greene. It is not the desire of the writers of this paper to interpose their judgment as to the taxonomic position of the species mentioned. It was thought, however, that a knowledge of gametophytic morphology might be of assistance when uncertainty and difference of opinion occurs.

A survey of the literature indicates that but little intensive work has been done on development. Guérin (1917) wrote very briefly on the microsporangium and the origin of the microsporocytes. Billings (1909) described the female gametophyte, young endosperm and embryo in the American species, *Salvia azurea* Lam. and *S. lanceolata* Willd. Schnarf (1917) described the nature of the female gametophyte and endosperm development in the Old World species, *S. pratensis* L. and *S. glutinosa* L.

The type of female gametophyte in the *Salvia* species figured by Schnarf (1917) differed markedly from that described by Billings (1909) in the two species he investigated. One purpose of the present study has been to shed light on the matter of form of the female gametophyte in *Salvia* by adding information with respect to six

additional species. Another purpose is to contribute new observations on microgametophyte development.

Pre-fertilisation material was fixed in Carnoy's fluid because of its excellent penetration. Nitrocellulose was used as an embedding medium, and iron-alum-haematoxylin as a stain. Post-fertilisation material was fixed in chrom-acetic acid, embedded in paraffin, and stained in iron-alum-haematoxylin or safranin.

MICROSPOROGENESIS IN *SAVIA MELLIFERA*

The archesporium is developed from a hypodermal layer that forms an inner sporogenous and an outer parietal layer. Through division by periclinal walls the parietal layer differentiates the tapetum. In a cross-section of the anther, the inner tapetal cells are found to be much elongated radially, while the outer ones are elongated tangentially. All are binucleate (Fig. 1). At the time of maturity of the microsporocytes the inner tapetal cells are filled throughout with cytoplasm, but by the time the microspore quartets are forming, the end directed toward the quartets becomes vacuolated, thus imparting a glandular appearance to this portion of the tapetum. A similar appearance has been noted by Coulter and Chamberlin (1903) in *Scrophularia nodosa*.

In diakinesis a large nucleolus is present and in Fig. 2, 15 bivalent chromosomes are visible. A polar view of metaphase of I shows the n count to be definitely 15 (Fig. 3). The chromosomes as seen in polar and side views in metaphase of I are spherical or elliptical in outline and show a slight variation in size. Metaphase appears to be quite regular, that is, with no laggards in reaching the equatorial plate (Fig. 4); but beginning with anaphase in which normal disjunction takes place (Fig. 5) certain peculiarities become manifest, the most prominent of which is the tendency of some of the chromosomes to precede others in passing to the poles. In fact a stage in anaphase is reached in which the daughter chromosomes are fairly well distributed over the spindle (Fig. 6). Instances were observed in which a single bivalent would reach the poles before disjunction was well started. Whether disjunction of this bivalent occurred subsequently or whether it entered the daughter nucleus as a bivalent was not determined. It is possible for disjunction to occur, however, when it is at least halfway to the pole. In these cases a diminutive spindle forms (Fig. 7). Chromosomes separating on the equatorial plate do not show the connecting diminutive spindles, the observed

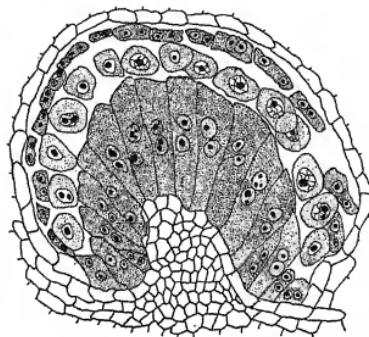


Fig. 1.



Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.

Fig. 1. *S. mellifera*. Cross-section of anther showing inner elongated tapetal cells and single layer of microsporocytes. $\times 300$.

Fig. 2. *S. mellifera*. Diakinesis, showing 15 bivalents. $\times 300$.

Fig. 3. *S. mellifera*. Metaphase of I showing an *n* count of 15. $\times 650$.

Fig. 4. *S. mellifera*. Normal metaphase of I. $\times 650$.

Fig. 5. *S. mellifera*. Disjunction. $\times 650$.

Fig. 6. *S. mellifera*. Irregular anaphase of I. 30 chromosomes on the spindle. $\times 650$.

instances being seen midway to the poles. Telophase shows the spindle quite cleared of chromosomes between the poles.

Interphase shows two elongated, often curved nuclei with the convex surface turned away from the centre. Chromosomes apparently unchanged from the telophase are present, also nucleoli. The cytoplasm often contains chromatin-like granules (Fig. 8). Following interphase the nuclei elongate, and a few prominent spindle fibres appear, along which the chromosomes tend to line up single file. The spacing of the chromosomes in this prophase is such that a count is easily possible, the number being 15 (Figs. 9, 10). Bleier (1933) found similar instances of chromosome spacing in *Oenothera franciscana*, but he calls it an early telophase, since equational separation has taken place. He thinks the spacing is due to some injury of the cell by which the movement of the chromosomes is hindered. In *Salvia mellifera* many of the chromosomes have already begun equational division, as is indicated by their constricted form. Metaphase appears quite normal (Fig. 11), but late phases are sometimes accompanied by many darkly stained particles resembling chromatin in the cytoplasm (Fig. 12). The origin of these particles is unknown. Nuclei in the quartet stage closely following telophase are considerably elongated, often crescent shaped, and contain several nucleoli (Fig. 13).

Microspore formation is simultaneous and is accomplished by true furrowing which commences in the late anaphase of II (Fig. 12). Furrowing microsporogenesis among angiosperms has been reported by several investigators, among whom are Andrews (1902), who found it in *Magnolia* and *Liriodendron*; Castetter (1925), who found it in *Melilotus alba*; Farr (1918), who found it in *Magnolia*; Denham (1924) in cotton; and Maneval (1914) in *Magnolia*. Farr (1918) has called attention to the increase in number of similar instances of furrowing or quadripartition in microsporogenesis of higher plants, which have been revealed by recent researchers. It may be stated that in general the process begins after the completion of the second nuclear division in meiosis, and is not accompanied by cell plate formation. Some variations in the details of the division have been recorded. Farr (1918) reports that furrows in *Melilotus* follow a definite course from the invaginations on the microspore toward the centre of the microspore to form the four quartets. Castetter (1925) reports a different furrowing process in that the furrow follows a chain of vacuoles, and that a special wall surrounds for a time the quartet of microspores. Neither vacuoles nor special walls were seen

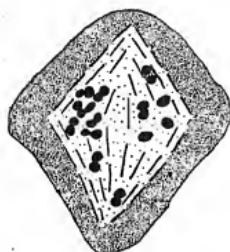


Fig. 7.

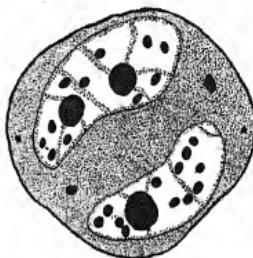


Fig. 8.

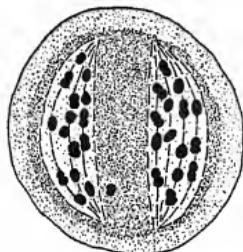


Fig. 9.



Fig. 10.

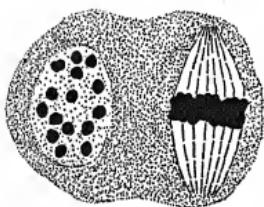


Fig. 11.

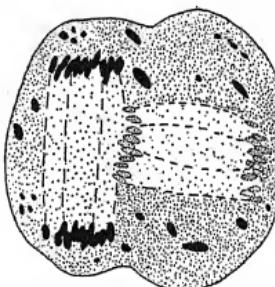


Fig. 12.

Fig. 7. *S. mellifera*. Irregular anaphase of I in which a bivalent has nearly reached a pole, while another is undergoing disjunction with a diminutive spindle. The entire chromosome complement is not shown. $\times 650$.

Fig. 8. *S. mellifera*. Interphase showing curved nuclei and disjoined chromosomes. $\times 650$.

Fig. 9. *S. mellifera*. Prophase of II showing chromosomes tending to follow certain spindle fibres. 15 chromosomes on each spindle. $\times 650$.

Fig. 10. *S. mellifera*. View of a spindle in prophase of II taken at right angles to the section shown in Fig. 9. 15 chromosomes present. $\times 650$.

Fig. 11. *S. mellifera*. Metaphase of II. 15 chromosomes. $\times 650$.

Fig. 12. *S. mellifera*. Telophase of II showing chromatin-like particles in the cytoplasm and the first stage of furrowing. $\times 650$.

by other investigators; nor do either of these features accompany the furrowing in *Salvia mellifera*. In the species described in the literature listed above there develops an additional spindle, so that the nuclei of the group of three seen in one plane in the tetrahedral arrangement are all connected. Additional spindles are absent in the quartets in *S. mellifera*.

A late stage in furrowing is represented in Fig. 14, in which one pair of developing microspores is superposed over the other pair in such a manner that they exhibit a decussate arrangement which is the result of the spindles in II forming in directions at right angles to each other (Fig. 12). The decussate grouping in microspore quartets has been described by Farr (1918) in *Magnolia*, and by Billings (1934) in *Atriplex hymenelytra*. A case of furrowing which results in the tetrahedral arrangement is shown in Fig. 15.

Small dark bodies which probably represent the chromatin extruded in meiosis are found in all young microspores (Figs. 12, 13), but by the time they are matured the chromatin matter has mostly disappeared. Ripe anthers contain two-celled microspores with a very low percentage defective.

Irregularities in meiosis such as chromosome lagging and chromatin extrusion are apparently not so uncommon as was once supposed. Because such phenomena occur in known hybrids, the parent plants of which are free from them, it is believed that hybridism is responsible for their appearance. According to Munz (1927) *Salvia mellifera*, of which the type form is abundant about Redlands, does hybridise with *S. apiana*, another well-marked species; but the plants from which the material for this paper was collected formed a somewhat isolated clump of undoubted type form. This does not mean, however, that hybridisation has not occurred at some more or less remote period.

S. mellifera shows a somatic count of 30 (Fig. 16), the chromosomes in which are longer than those seen in meiosis.

MICROSPOROGENESIS IN *SALVIA APIANA*

Beginning with the early prophase, the nucleolus in the microsporocyte begins a process of budding, the first bud to be formed reaching a size nearly the size of the main portion of the nucleolus, from which it finally becomes detached (Fig. 17). Additional buds originate, but while they do not reach as large a dimension as the first bud, they separate from the main mass as the above did by constriction (Fig. 18). In the meantime synapsis is reached, when

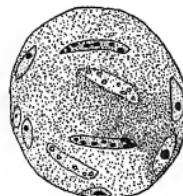


Fig. 13.



Fig. 16.

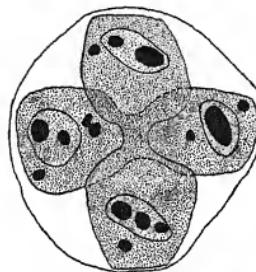


Fig. 14.



Fig. 17.

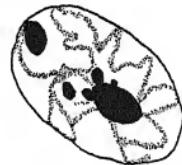


Fig. 18.

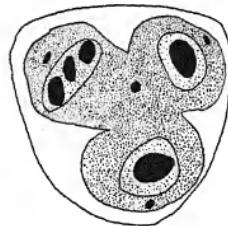


Fig. 15.



Fig. 19.

Fig. 13. *S. mellifera*. Quartet of nuclei showing crescent shape and prominent nucleoli. Chromatin-like granules in the cytoplasm organised as micro-nuclei. $\times 650$.

Fig. 14. *S. mellifera*. Late stage of furrowing with decussate arrangement of microspores. $\times 650$.

Fig. 15. *S. mellifera*. Furrowing with tetrahedral arrangement of microspores. $\times 650$.

Fig. 16. *S. mellifera*. Somatic metaphase showing 30 chromosomes. $\times 650$.

Fig. 17. *S. apiana*. Diagram illustrating formation and abstraction of nucleolar buds.

Fig. 18. *S. apiana*. Budding in a microsporocyte. $\times 650$.

Fig. 19. *S. apiana*. Synapsis, in which nucleolar buds are abstracted into the nuclear knot. $\times 650$.

the buds formed are found to arise on the side of the nucleolus facing the condensed tangle of chromatin threads (Fig. 19), among which may be found detached buds of earlier origin. The budding process continues into diakinesis, at which time the bivalents, nucleolus and buds may be seen enclosed within a broken nuclear wall (Fig. 20), from which some of the extruded chromatin and nucleolar matter has passed out into the cytoplasm. Nucleolar budding in *S. apiana* appears to be a regularly occurring feature in microsporogenesis, since it was observed without exception in the many anthers examined. It is not peculiar to this species however, for it has been reported in *Acer platanoides* by Cardiff (1906), in *Melilotus alba* by Castetter (1925), in *Acer negundo* by Darling (1909), in the cotton plant by Denham (1924), in *Smilax herbacea* by Elkins (1914), and in *Phaseolus* by Wager (1904).

The fate of the nucleolar buds, of which, it seems, there may be several, probably varies in the different cells and probably also is related to the purpose for which they have arisen. Satisfactory answers to these questions are not as yet available. As to fate, it appears that at the dissolution of the nuclear wall the buds at least may escape into the cytoplasm and thus explain the presence of certain darkly staining granules found there during later stages in meiosis (Figs. 21, 22). In a few cases they have escaped from the cytoplasm and become walled off on the outside of the cell (Figs. 21, 22). Such appearances as those seen in the figures just mentioned would lead one to suppose that the material composing buds is of extraneous nature, the extrusion of which is undertaken by the cell.

Instances of nucleolar budding within somatic cells have been found in *Salvia mellifera* in the terminal cells of glandular trichomes. The detached portions pass to the periphery of the nucleus where, due to a lighter zone about them, they appear as micronuclei. Such may be seen in various stages of passage through the nuclear membrane to the cytoplasm (Fig. 22).

Tischler (1921-2, p. 324), in a discussion of budding nucleoli, is of the opinion that the phenomenon is an artifact of fixation, since the tendency of proper fixation is to impart a spherical form to nucleoli. After witnessing buds in all degrees of development and finally abstracted and lying in the vicinity of the nucleolus, it is difficult to accept the idea that they are merely artifacts. It is certain that most nucleoli do not exhibit budding, so the question naturally suggested is: Why should not the same fixing fluid act accordingly on all nucleoli in the same material?

In Fig. 21, showing diakinesis, the nucleolus has disappeared, 15 bivalents only remaining within the nucleus. A rupture in the nuclear wall probably indicates the spot through which one or more nucleolar buds entered the cytoplasm.

Cytomixis is not a constant phenomenon in the development of the microspores, but has been found in a few instances when it appeared during diakinesis (Fig. 23). A conspicuous feature is the behaviour of the nucleolus which extends as a slender process from the nucleus of one cell to the cytoplasm of an adjacent cell. The process occupies the centre of a bridge that is formed by the nuclear wall. Darkly stained particles surround the base and tip of the nucleolus. Some bivalents lie well back within the nucleus. It will be observed that the nuclear wall penetrates the walls of both cells between which cytomixis occurs. An explanation of the cause of such a peculiar condition as that seen in Fig. 23 is left for a later discussion, but there occur some post-cytomixistic stages in *S. apiana* that preclude the idea, so far as this species is concerned, that the phenomenon is the result of fixation or injury due to micro-technique. There is a partial recovery from cytomixis, various stages in which have been found, and which are represented in Figs. 24-26. The first act is the abstraction of the nucleolar process, or at least an elimination of the portion that extends beyond the cell wall in an extra-cellular position. Such material may consist not only of nucleolar substance, but also of darkly stained granules of uncertain origin. There does not appear to be any loss of cytoplasm through the line of contact between nuclear wall and cell wall. In some instances the main mass of the nucleolus lies fairly at the mouth of the opening of the nucleus to the outside (Fig. 27). It is probable that the stages seen in these latter two figures are some that are further advanced toward recovery. Later stages consist in gradual retraction of the nucleolar process, with ultimate reconstruction of both nuclear and cell membranes (Fig. 26).

Cytomixis in *S. apiana* appears to be an abnormality throughout. This is shown in part by the interference of normal diakinesis in which the number of bivalents characteristic of the species is altered. There is a tendency of the chromatin to gather in groups of four granules, or else in *Streptococcus*-like chains. The group of darkly stained particles that hover about the base of the nucleolus may be accounted for on the basis of a fragmentation of chromosomes. It is unlikely that microsporocytes which have thus far recovered from the cytomixistic state are in a position to continue meiosis in a normal manner.

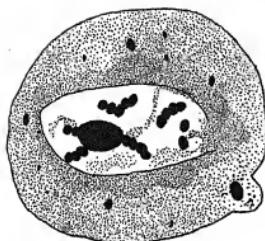


Fig. 20.

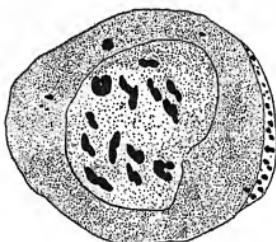


Fig. 21.

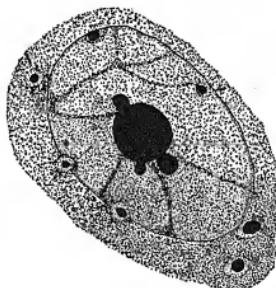


Fig. 22.

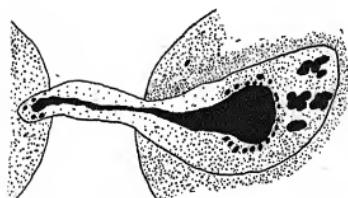


Fig. 23.

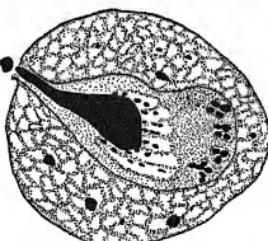


Fig. 24.

Fig. 20. *S. apiana*. Diakinesis, in which either extruded chromatin, or nucleolar buds, or both, are found in the cytoplasm. The nuclear wall is broken, and what appears to be a bud is in the process of being eliminated from the cell. $\times 650$.

Fig. 21. *S. apiana*. Diakinesis. Extruded chromatin or nucleolar substance has been walled off and has undergone partial disintegration. $\times 650$.

Fig. 22. *S. mellifera*. Nucleolar budding in the terminal cell of a glandular trichome. The buds appear to be organised as micronuclei and are passing through the nuclear membrane into the cytoplasm. $\times 650$.

Fig. 23. *S. apiana*. Cytomixis. An extension of the nucleolus surrounded by the nuclear membrane extends from one microsporocyte to another. $\times 650$.

Fig. 24. *S. apiana*. Cytomixis. Abstriction of the extracellular portion of the nucleolus. $\times 650$.

The literature covering instances of cytomixis is now considerable. The nucleolus is not always the part of the nucleus that extends from one cell to the other, nor is there always a slender tube-line extension between the reaching cells. In *Alnus corylus*, according to Woodworth (1929), chromosomes extend from or between the cells in close contact. In *Scirpus*, as reported by Hicks (1928), the nucleolus extends a pointed process between the cells, something after the order of *Salvia apiana*. In *Scirpus* also the chromosomes may connect across the cells by filamentous extensions. In this genus the cells are shown to be in close contact.

Church (1929) reports cytomyxis as not uncommon in the grasses, and that the condition is attended by ejection of chromatin from the nucleus of one microgametocyte to the cytoplasm of the adjacent cell, and hence the species is not so good an illustration of cytomixis according to the definition first propounded by Gates (1908). According to Church (1929) the nucleolus is concerned in cytomixis, for Digby (1909) reported ejections of nucleolar material in *Galtonia candicans*.

The physical cause of cytomixis has been a subject for proposed explanations, such as "disturbance in electric equilibrium" (Farr, 1918), and difference in turgor pressure (Tischler, 1921-2, p. 177). That it is found in known hybrids, and in plants in which hybridism is suspected because of other abnormalities during meiosis, is now well known. Understood in this light cytomixis is an abnormality which with certain other irregularities soon to be mentioned indicates that hybridism has occurred at some time in the history of *Salvia apiana*.

Metaphase of I in *S. apiana* shows that the bivalents are regularly arranged on the equator which when observed from the poles gives the n chromosome count of the species as 15 (Fig. 29). As the basic number for the Labiateae is probably 8 (Wanscher, 1934), *S. apiana* may be considered a hypoploid. In anaphase, normal disjunction generally occurs, and without lagging (Fig. 28). Exceptions, however, are found in which non-disjunction takes place, with bivalents more or less scattered over the spindle (Fig. 30), and later appearing in the interphase (Fig. 31). Following interphase the nuclei elongate, assuming a spindle form much as in *S. mellifera*. Prominent spindle fibres appear, with some of the chromosomes in single file upon them (Fig. 32). Metaphase is regular, though chromatin-like bodies are found in the cytoplasm (Fig. 33).

Furrowing occurs in *S. apiana* beginning with anaphase of II, but

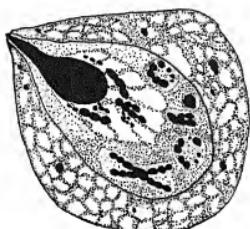


Fig. 25.

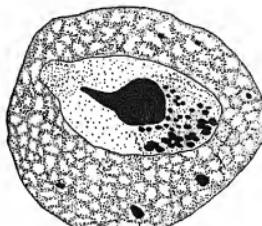


Fig. 26.

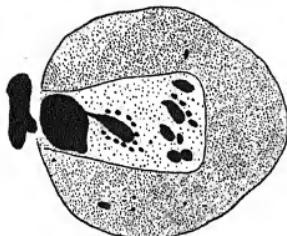


Fig. 27.

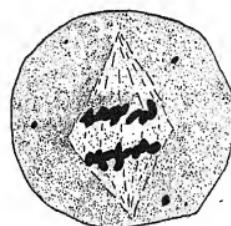


Fig. 28.

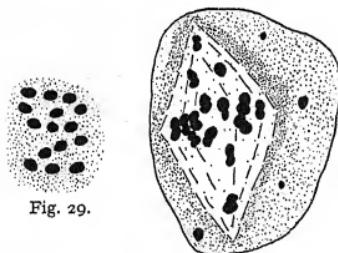


Fig. 29.

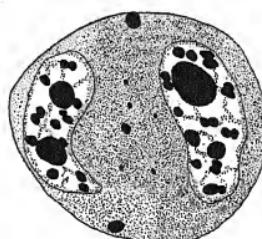


Fig. 30.

Fig. 25. *S. apiana*. Cytomixis. The cell wall has closed the opening through which nuclear membrane and nucleolus protruded. $\times 650$.

Fig. 26. *S. apiana*. Cytomixis. Late stage in recovery. $\times 650$.

Fig. 27. *S. apiana*. A stage in cytomixis occasionally seen. $\times 650$.

Fig. 28. *S. apiana*. Regular anaphase.

Fig. 29. *S. apiana*. Metaphase of I. 15 chromosomes. $\times 650$.

Fig. 30. *S. apiana*. Irregular anaphase with bivalents passing to the poles. $\times 650$.

Fig. 31. *S. apiana*. Interphase. A few bivalents are seen in each nucleus. $\times 650$.

differs in certain details from the process as observed in *S. mellifera*. In the latter species the first signs of the furrows are external invaginations, while in *S. apiana* invagination is accompanied by furrowing in the centre of the quadrinucleate cell (Fig. 34).

The function of vacuoles in furrowing was first noticed by Castetter (1925) in *Melilotus alba*, in which a series of vacuoles fuse along the lines of furrowing. In *Salvia apiana* a single central vacuole extends centrifugally to meet the invaginations.

Arrangement of the microspores may be either decussate (Fig. 35) or tetrahedral, depending on spindle direction.

The mature microgametophyte is two-celled, with cytoplasm quite free from extruded chromatin-like material.

The somatic chromosome number in *S. apiana* was ascertained to be 30 (Fig. 36).

DEVELOPMENT OF THE MEGAGAMETOPHYTE

The megasporocyte in *S. mellifera* gives rise to an axial row of four megasporangia which are surrounded by a single layer of nucellus cells (Fig. 37). The innermost megasporangium is the functional one which develops an eight-celled female gametophyte. Schnarf (1917) found that the innermost megasporangium was the functional one in *S. glutinosa* and *S. pratensis*.

The mature megagametophyte was observed in *S. splendens*, *S. greggii*, *S. leucantha*, *S. mellifera*, *S. apiana* and *S. columbariae*. Two entirely different types of megagametophytes are found among the six species mentioned, one type being characteristic of the former three species and a second type characteristic of the latter three. The first will be designated as the *S. splendens* type. The megagametophyte of *S. splendens* is little more than twice as long as wide, and occupies only a small portion of the lower part of the ovule as seen in a medial longitudinal section passing through the funiculus and the micropyle. In this section, one side of the megagametophyte is strongly convex, the opposite side slightly concave (Fig. 38). The egg apparatus and primary endosperm nucleus are relatively large. The chalazal end, containing three antipodal nuclei, is directed toward the terminus of the vascular bundle that enters the ovule and passes downward through the integument. There is a complete absence of an epithelium (to use Goebel's (1905) term for the differentiated integumental cell layer that surrounds the megagametophyte). The megagametophytes of *S. leucantha* and *S. greggii* vary from that of *S. splendens* in no important detail (Figs. 39 and 40).

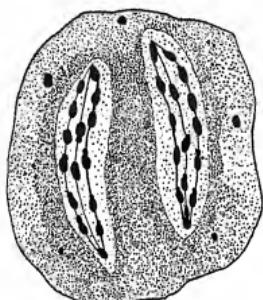


Fig. 32.

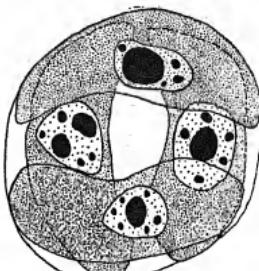


Fig. 35.

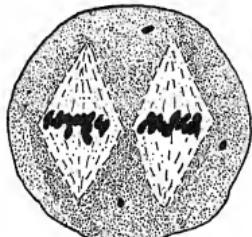


Fig. 33.

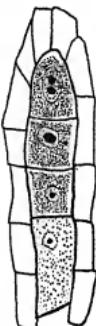


Fig. 37.

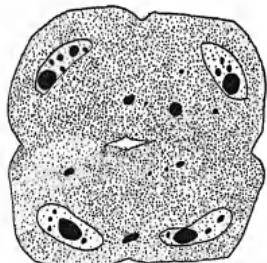


Fig. 34.



Fig. 38.

Fig. 32. *S. apiana*. Typical prophase of II, in which the chromosomes tend to lie single file on certain prominent spindle fibres. $\times 650$.

Fig. 33. *S. apiana*. Normal metaphase of II. $\times 650$.

Fig. 34. *S. apiana*. Quartet of microspore nuclei with beginning of furrowing. $\times 650$.

Fig. 35. *S. apiana*. Decussate arrangement of microspores. $\times 650$.

Fig. 36. *S. apiana*. Somatic metaphase. 30-chromosomes. $\times 650$.

Fig. 37. *S. mellifera*. Axial row of megaspores. $\times 650$.

Fig. 38. *S. splendens*. Mature megagametophyte. $\times 300$.

The type of the three species, *S. mellifera*, *S. apiana* and *S. columbariae*, will be designated as that of *S. mellifera*. This megagametophyte is much elongated, is slightly or not at all curved, and at maturity is divided into two distinct portions (Fig. 41). The lower and longer part is narrow or tubular, and is surrounded by an epithelial layer. The endosperm nucleus lies near the upper portion of this, while at its lower end the three antipodals are found. The upper or micropylar portion is wider and shorter, without bordering epithelium, and is approximately two-thirds the length of the entire megagametophyte in *S. splendens*. The megagametophytes of *S. apiana* and *S. columbariae* are similar to that of *S. mellifera* (Figs. 42 and 43).

Billings (1909) investigated the megagametophytes of the American species, *S. azurea* and *S. lanceolata*. They are of the *S. splendens* type. Schnarf (1917) worked with the European species *S. pratensis* and *S. glutinosa*, which are of the *S. mellifera* type. Schnarf (1917) was perplexed at the wide diversity in form in the species with which he worked and those described by Billings, and urged further investigation. At first sight it might have appeared as though the European and American species differed in accordance with geographical location. It was partly to clear up the matter that additional species were investigated by the authors. That none but New World species was selected was because no material of Old World forms was available. The results of the study of the six species described herein indicates that at least two distinct type forms of megagametophyte are found in *Salvia*, that both are found in the Americas, and that Billings merely happened to work with one type only. It can only be conjectured whether both are found in the Old World, but it is probable that further investigation will demonstrate the presence of the *S. splendens* type.

POST-FERTILISATION DEVELOPMENT

In the *S. splendens* type the spindle of the first division of the endosperm nucleus is longitudinal, with the result that one daughter nucleus occupies the chalazal end, in which it becomes separated from the other (micropylar) nucleus by a transverse wall (Fig. 44). The chalazal endosperm nucleus divides once more only, the resulting nuclei with the surrounding cytoplasm developing into a haustorium that elongates in the direction of the terminus of the vascular bundle (Fig. 45).

The upper or micropylar nucleus resulting from the first division of the primary endosperm nucleus undergoes a division in which



Fig. 39.

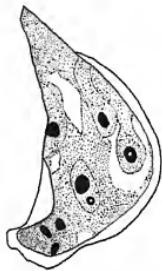


Fig. 40.



Fig. 41.



Fig. 42.

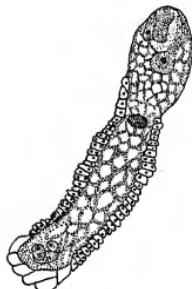


Fig. 43.

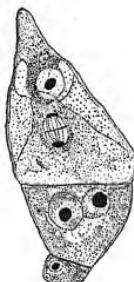


Fig. 44.

Fig. 39. *S. leucantha*. Mature megagametophyte. $\times 300$.

Fig. 40. *S. greggii*. Mature megagametophyte. $\times 300$.

Fig. 41. *S. mellifera*. Mature megagametophyte. $\times 300$.

Fig. 42. *S. apiana*. Mature megagametophyte. $\times 300$.

Fig. 43. *S. columbariae*. Mature megagametophyte. $\times 300$.

Fig. 44. *S. splendens*. Young endosperm. The transverse wall was formed after the first division of the primary endosperm nucleus. Uppermost nucleus is that of the zygote; lowermost, an antipodal. From the dividing nucleus one daughter begins the organisation of the micropylar haustorium, the other develops endosperm tissue. $\times 300$.

the spindle is longitudinal. Of the two daughter nuclei thus formed, the one nearer the micropyle begins the organisation of a haustorium, while the other originates the endosperm tissue in which the embryo develops. Thus three distinct portions differentiate, a micropylar haustorium, a central endosperm tissue and a chalazal haustorium. The micropylar haustorium (Fig. 45), like the chalazal, finally contains two nuclei, and extends into the adjacent integument, first as an elongated outgrowth, later as a hemispherical structure. Enlargement of the endosperm tissue soon forces it to a lateral position (Fig. 46).

The zygote develops a short suspensor that pushes the pro-embryo into the young endosperm tissue. The suspensor elongates also in the opposite direction until a portion of it comes to lie within the developing micropylar haustorium (Fig. 46). While both haustoria may be said to collect food ultimately for the young embryo, the immediate recipients of such food are the embryo in the case of the micropylar, and the endosperm in the case of the chalazal haustorium.

The endosperm tissue extends rapidly upward through the integument, at first in the form of a long process (Fig. 47). Lateral growth of this tissue eventually effects the obliteration of the micropylar haustorium.

Post-fertilisation development in the *S. splendens* type resembles, in general, that found in *S. lanceolata* and *S. azurea* described by Billings (1909). According to his figures, however, the micropylar haustorium in both species is finally much elongated, which is a variation from the approximately hemispherical haustorium of *S. splendens*, *S. greggii* and *S. leucantha*. In *S. azurea* three nuclei are seen in the haustorium, while in *S. lanceolata*, as in *S. splendens*, only two are present.

In *S. mellifera* quite a different post-fertilisation history from that of the *S. splendens* type would be expected because of such radical variation in form of megagametophyte. The endosperm nucleus in *S. mellifera* lies at fertilisation within that portion of the megagametophyte bounded by epithelium. The first division spindle is longitudinal, the micropylar daughter nucleus passing into the upper part of the megagametophyte, or that which is without bounding epithelium. Here it undergoes one division (Fig. 48). This binucleate portion can be designated as a micropylar haustorium, which has a counterpart in species of different plant families. Among the Labiatae such a haustorium has been described by Billings (1909)

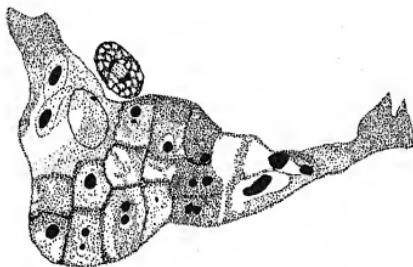


Fig. 45.

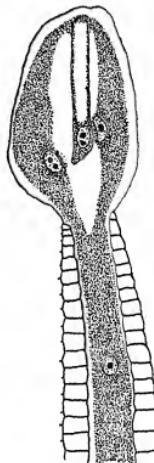


Fig. 48.

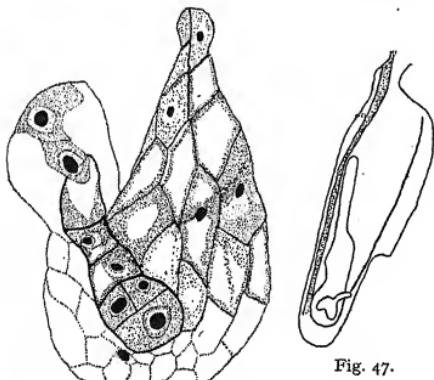


Fig. 46.

Fig. 47.

Fig. 45. *S. splendens*. Young endosperm, micropylar and chalazal haustoria. The dividing zygote (drawn outside) lies below the micropylar haustorium. Its location is indicated by the oval. $\times 300$.

Fig. 46. *S. splendens*. Endosperm, embryo and micropylar haustorium, the last becoming lateral because of the beak-like extension of endosperm tissue. $\times 300$.

Fig. 47. *S. splendens*. Diagram of ovule showing embryo in young cotyledon stage embedded in the basal part of the elongated endosperm. The dark line extending from the suspensor is the micropylar canal. The shaded portion is the vascular bundle. $\times 300$.

Fig. 48. *S. mellifera*. Two-celled micropylar haustorium containing an elongating suspensor. Epithelium bounds the main portion of the megagametophyte in which is seen one of the daughter nuclei resulting from the first division of the primary endosperm nucleus. $\times 284$.

in *Lamium amplexicaule*, *Stachys palustris*, *Leonurus cardiaca*, *Nepeta cataria*, by both Billings (1901) and Sharp (1912) in *Physostegia virginica* and by Schnarf (1917) in *Stachys sylvatica*, *Salvia pratensis*, *S. glutinosa*, *Ajuga repens*, *Brunella vulgaris*, *Satureja vulgaris* and *Mentha austriaca*. The haustoria vary in growth extension in different species, but in some, however, activity is greater. In *Salvia mellifera* there is a nearly uniform enlargement over the entire surface with corresponding destruction of integument cells.

The lower of the two daughter nuclei that result from the division of the primary endosperm nucleus undergoes a longitudinal division, the nucleus nearest the chalaza passing into the lower end of the tubular portion of the megagametophyte and there organising a chalazal haustorium. By a division of its nucleus it soon becomes binucleate (Fig. 49). The empty appearance of the cells immediately surrounding the distal end of the haustorium attests the activity with which it absorbs food materials.

Fertilisation is followed by an extensive elongation of the suspensor without cell formation in its upper portion. The pro-embryo is thus brought well down into the young endosperm which is developing within the central portion bounded by epithelium (Fig. 50). The epithelial layer is here seen to have become two cells thick over most of its length.

Post-fertilisation development in *S. mellifera*, *S. apiana* and *S. columbariae* finds an approximate parallel in *Pentstemon secundiflorus*, described by Evans (1919), and in the various Labiateae listed above as reported by Billings (1909) and Schnarf (1917). In all these the megagametophyte differentiates into at least two portions: first, an upper or micropylar part containing the egg apparatus and later the upper portion of the long suspensor; and, secondly, a lower or chalazal part in which endosperm tissue and embryo develop. A third portion or chalazal haustorium is also found in most if not all of the species referred to above. The endosperm tissue is soon partly cut off from the micropylar part above by a constriction (Fig. 50).

When *Salvia* species of the *S. splendens* type are compared with those of the *S. mellifera* type, some notable variations are evident. The suspensor in the *S. splendens* type is short, with cells that appear active and that function as a connection with a haustorium in the nutrition of the embryo. There is no epithelium in the *S. splendens* type, so that there is an early development of endosperm tissue as an outgrowth into the integument. An epithelium is present in the *S. mellifera* type and it tends to confine endosperm development.

In this type the suspensor elongates greatly without cell formation except in the lower portion, and does not appear to function in connection with the haustorium. While both types have micropylar and chalazal haustoria, the micropylar in the *S. mellifera* type remain directly between the lower end of the micropylar canal and the upper portion of the advancing endosperm until obliteration occurs; while in the *S. splendens* type the micropylar haustoria soon become lateral with respect to the endosperm because of the extension of this tissue to one side of, though approximately parallel with, the micropylar canal (Fig. 47).

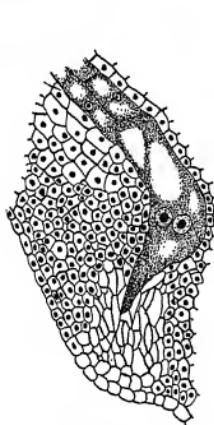


Fig. 49.



Fig. 50.

Fig. 49. *S. mellifera*. Chalazal region of ovule showing two-celled chalazal haustoria. $\times 284$.

Fig. 50. *S. apiana*. Two-celled micropylar and chalazal haustoria between which is the cellular portion of the endosperm with a young embryo. Constriction at the upper portion of the epithelium. $\times 284$.

DEVELOPMENT IN RELATION TO SYSTEMATIC POSITION

Engler and Prantl (1897) place *S. mellifera* and *S. apiana* in the genus *Ramona* under the specific names of *R. stachyoides* Briquet and *R. polystachya* Greene respectively. Munz (1927), in his revision of *Salvia*, appears doubtful of maintaining the genus *Ramona*, and finally decides not to recognise it. The three species *Salvia mellifera*, *S. apiana* and *S. columbariae* have a similar megagametophytic

history. *S. columbariae* has been uniformly placed in *Salvia*; the other two species in *Salvia* by some taxonomists, in *Ramona* by others. The two genera have been differentiated taxonomically as follows. *Salvia*: upper corolla lip erect; connective of filaments transverse, lower portion evident. *Ramona*: upper corolla lip spreading; connective of filament continuous with it, lower portion not evident or indicated by a tooth.

The species of *Salvia* (excluding those in *Ramona*) in which the form of the megagametophyte has been determined, are classified into subgenera, according to Engler and Prantl (1897), as follows:

<i>S. glutinosa</i>	Subgenus <i>Salvia</i> (all Old World species)
<i>S. pratensis</i>	Subgenus <i>Sciaraea</i>
<i>S. columbariae</i>	Subgenus <i>Leonia</i>
<i>S. lanceolata</i>	Subgenus <i>Jungia</i>
<i>S. azurea</i>	Subgenus <i>Jungia</i>
<i>S. leucantha</i>	Subgenus <i>Jungia</i>
<i>S. greggii</i>	Subgenus <i>Jungia</i>
<i>S. splendens</i>	Subgenus <i>Jungia</i>

The five listed in subgenus *Jungia* are all New World species, and the same five have a similar type of megagametophyte (*Salvia splendens* type). The two species *S. polystachya* and *S. mellifera*, placed in the genus *Ramona* by Engler and Prantl, have a megagametophyte similar to *Salvia glutinosa*, *S. columbariae* and *S. pratensis* (*S. mellifera* type). While, as stated above, but little work has been done on the developmental history of the species of *Salvia*, which is a large genus, knowledge thus far gained would seem to afford more reason for placing the listed species of subgenus *Jungia* into a separate genus because of their similar and unique development than it would for placing in separate genera the three species *Salvia columbariae*, *S. glutinosa* and *S. pratensis* on the one hand, and on the other the two species *S. mellifera* and *S. apiana*, the embryogeny of all five of which follow a similar course.

CHROMOSOME NUMBERS

Chromosome counts of *Salvia* species that are available are as follows:

	<i>n</i>	<i>2n</i>
<i>S. nipponica</i>	8	—
<i>S. columbariae</i>	16	32
<i>S. splendens</i>	16	—
<i>S. leucantha</i>	16	—
<i>S. apiana</i>	15	30
<i>S. mellifera</i>	15	30

The basic or monoploid chromosome number is probably 8, though this conjecture rests on a single form, *S. nipponica*, worked on by Morinaga (1930). Of the species reported on in this paper there are three tetraploid, namely *S. columbariae*, *S. splendens* and *S. leucantha*. Two others are hypoploid, *S. apiana* and *S. mellifera*, these evidently having been derived from tetraploidy by loss of a chromosome during meiosis. Counts were not obtained for *S. greggii*.

SUMMARY

1. Details in development were studied in *Salvia mellifera* and *S. apiana*, but four other species were examined especially for their type of female gametophyte.

2. Several unusual features are associated with the process of microsporogenesis in *S. mellifera* and *S. apiana*, which may be briefly described as follows:

(a) Nucleolar budding occurs in *S. apiana* during meiosis, the buds arising and constricting much as those in yeast. There is no evidence that they are definite chromosomes, though they may be of chromatin material and contribute to the formation of chromosomes. They may be extruded and thus account for some at least of the darkly stained granules appearing in the cytoplasm.

(b) Cytomixis is an abnormality appearing in diakinesis of *S. apiana*. The nucleolus is a conspicuous participant, and may extend as a slender process between two cells not in contact. A partial recovery of the microsporocytes occurs, which indicates that cytomixis is not an artifact of micro-technique.

(c) Metaphase in I is normal in both species but anaphase may be accompanied by non-disjunction of some of the bivalents in *S. mellifera*, with their appearance near the poles and in interphase. In *S. apiana* non-disjunction may occur, but is a comparatively rare abnormality.

(d) Prophase in II is attended by nuclear elongation and conspicuous spindle fibres (probably artifacts) along which the chromosomes appear in approximately single file.

(e) True furrowing occurs in both species of *Salvia* studied, in *S. mellifera* the furrows extending centrifugally, without accompanying central vacuolation. In *S. apiana* a central vacuole assists invagination.

(f) Microspore quartets may be decussate or tetrahedral in arrangement, depending on the direction of the spindles in II.

(g) The irregularities in meiosis suggest that hybridisation has occurred at some time in the history of the species.

3. The *n* count is as follows: *S. mellifera* 15; *S. apiana* 15; *S. splendens* 16; *S. leucantha* 16; *S. columbariae* 16. As the basic number of the Labiateae appears to be 8, *S. mellifera* and *S. apiana* may be considered hypoploids, *S. splendens*, *S. columbariae* and *S. leucantha* as tetraploids.

4. Two distinct forms of macrogametophytes occur:

(a) A short form without a bounding epithelium, found in *S. splendens*, *S. leucantha* and *S. greggii*, which is distinguished as the *S. splendens* type.

(b) A long form with a bounding epithelium extending about two-thirds the length of the macrogametophytes. This type is in *S. mellifera*, *S. apiana* and *S. columbariae*, and is designated as the *S. mellifera* type.

These types correspond with those previously observed by Billings (1909) and Schnarf (1917) respectively.

5. Suspensors are long, upper part evanescent, in those species that have the *S. mellifera* type of macrogametophyte. They are short and for a time persistent, and probably functional in embryo nutrition in the *S. splendens* type.

6. Micropylar and chalazal haustoria were found in all six species investigated.

ACKNOWLEDGMENT

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STRUCTURE AND DEVELOPMENT OF THE
SYNERGIDS IN *AMMANIA BACCIFERA* LINN.:
A CORRECTION

BY A. C. JOSHI AND J. VENKATESWARLU

STUDY of more species of *Ammania* and examination of more material of *A. baccifera* itself reveals that our previous account of the synergids of this plant (*New Phytol.* 34, No. 2 (1935)) is incorrect. It is based on a confusion with the endosperm. The real synergids are quite normal. They are pear-shaped structures with hooks and long apices. What have been described as fusing synergids in Fig. 2 are in reality very young just differentiating synergids. The endosperm develops much before the first division of the egg, and forms very dense accumulations both in the micropylar and chalazal regions of the embryosac. These are laterally connected only by very weak strands. During fixation the micropylar accumulation frequently breaks from these lateral strands and gets the distinct form of a collar surrounding the egg and later on the embryo. What has, therefore, been figured as a syn-synergid in Figs. 3-10 is in reality the micropylar accumulation of the endosperm. This is gradually absorbed during the development of the embryo. The mistake in our previous description occurred owing to the absence of normal synergid stages in the material we then examined, and the frequent presence of pollen-tubes with their contents intact close to the egg even after the development of the micropylar accumulation of the endosperm, such as is illustrated in Fig. 4.

[It is interesting to note that Figs. 45 and 46 (p. 85) of the paper by E. M. Carlson and B. C. Stuart in this volume are comparable with Figs. 3 and 6 referred to in the above correction, and show the micropylar cap of endosperm which was mistaken in *Ammania baccifera* for a fusion product of synergids. *Editorial note.*]

NOTE ON H. L. EDLIN'S PAPER ON
THE MALVALES¹

DR H. SLEUMER of the Botanischer Garten und Botanisches Museum, Berlin-Dahlem, in conversation during interludes between sessions at the Amsterdam Congress, called attention to some points of disagreement with certain conclusions reached by Mr Edlin in his paper on the Malvales published in the *New Phytologist* (1935); Dr Sleumer attributes these conclusions to the fact that Mr Edlin was not able to see and dissect for himself material of certain genera.

Dr Sleumer has kindly furnished the following summary of his observations:

We are of the opinion that parietal placentation does not exclude genera such as *Nettoa*, *Goethalsia*, and *Neotessmannia* from the Tiliaceae.

Prof. Burret has shown that *Nettoa* is identical generically with *Corchorus*; he found parietal placentation among various Australian species which are actually placed under *Corchorus* in Bentham's *Flora of Australia*. If *serial* sections are made, it is then seen that the placentation is parietal in the middle of the ovary only, and not in the upper and lower portions.

Some time ago, Gleason wanted to transfer the Tiliaceous genus *Goethalsia* to the Flacourtiaceae, on account of its parietal placentation, but had to admit that this is opposed by Burret, Rehder, Ducke and Standley in their publications.

Neotessmannia is definitely not Flacourtiaceae and has nothing to do with *Banara*, if only by reason of its inferior ovary and mucilage content.

If Mr Edlin had seen the plants, he would never have reached the view that they should be excluded from the Tiliaceae. That *Prockia* and *Hasseltia* belong to the Flacourtiaceae in spite of a certain resemblance to the Tiliaceae has long been established by Gilg: neither possesses mucilage cells.

¹ *New Phytol.* 34 (1935).

REVIEWS

Forest Trees and Timbers of the British Empire. Part I. *Some East African Coniferae and Leguminosae.* By L. CHALK, M.A., D.Phil., J. BURTT DAVY, M.A., Ph.D. and H. E. DESCH, B.Sc. $6 \times 10\frac{1}{2}$ in. Pp. 68, 10 pls., numerous text-figures. 1932. 5s. net. Part II. *Twenty West African Timber Trees.* By L. CHALK, M.A., D.Phil., J. BURTT DAVY, M.A., Ph.D., H. E. DESCH, B.Sc., M.A. and A. C. HOYLE, B.Sc., M.A. Pp. 108, 20 pls., numerous text-figures. 1933. 7s. 6d. net. Part III. *Fifteen South African High Forest Timber Trees.* By L. CHALK, M.A., D.Phil., M. M. CHATTAWAY, B.Sc., M.A., J. BURTT DAVY, M.A., Ph.D., F. S. LAUGHTON, B.Sc. and M. H. SCOTT, B.Sc. Pp. 103, 17 pls., numerous text-figures. 1935. 7s. 6d. net. All parts edited by L. CHALK and J. BURTT DAVY. Oxford: Clarendon Press.

"The magnitude of the task now begun by Dr Burtt Davy and Dr Chalk may appear overwhelming, but of its importance there can be no question." These words were written by Prof. R. S. Troup in the preface to Part I of *Forest Trees and Timbers of the British Empire*. The important task referred to consists primarily in combining taxonomic descriptions of the more important timber trees in the British Empire with detailed accounts of the macroscopic and microscopic structure of their wood. In addition the common, vernacular and botanical names of each tree are given, as well as notes on distribution, climatic conditions, vegetation type, regeneration, afforestation, diseases, and seasoning and working qualities of the wood. Forestry officers in the various localities where the trees occur co-operated in supplying the necessary material and such information as could only be obtained locally.

In the three parts of this work which have now been published the descriptions have been confined to certain trees, occurring in East, West, and South Africa respectively, which provide timber of economic importance or which are likely to be utilised on a larger scale in the future. In every instance efforts have been made to ensure that the descriptions are based on wood specimens and herbarium material from the same source. This is one of the most important features of the work and is, except for a few isolated instances, a new departure so far as descriptions of Empire timbers and the trees which produce them are concerned. Numerous difficulties arise when this type of correlation is attempted, as can be fully appreciated only by those with first-hand experience of this type of work. It is doubtless because accurately named material cannot readily be obtained that the authors have begun by describing a miscellaneous collection of trees from three different sources, rather than attempt an exhaustive survey of those from any one locality at a time. It is evident, however, that this lack of planning has disadvantages, which might be overcome if arrangements were made for material to be collected in good time.

The first part of this work was written at a time when there was no generally accepted technique for describing wood structure accurately, or of securing those measurements which are generally considered to be of diagnostic value. Nor were the descriptive terms in common use well defined. Considerable progress towards general agreement on these subjects has since been made through the "International Association of Wood Anatomists". In order to

incorporate the recommendations of this society, changes have been made in the technical terms employed as well as in the mode of presentation of the descriptions of the woods dealt with in succeeding parts of the work. It is, however, to be hoped that as few changes of this kind as possible will be introduced in future numbers so as to ensure uniformity throughout the work as a whole.

A feature of the work which greatly enhances its value is the illustrations. All of these are excellent, and include original line drawings of herbarium specimens, habit photographs of the trees, and photomicrographs of transverse and tangential sections of the wood.

Forest Trees and Timbers is intended primarily as a work of reference, and as such it will be found invaluable by those who are in any way interested in timber or forest trees. It is, however, to be hoped that other botanists than those who are primarily interested in forestry may find the book useful. In spite of the view that is sometimes expressed to the effect that anatomical investigations seldom do more than lead to an accumulation of facts of little value, it is, nevertheless, true that the existing literature on systematic anatomy (and especially on wood structure) is very inadequate and frequently misleading. For this reason *Forest Trees and Timbers* cannot fail to interest palaeobotanists who make use of anatomical features in elucidating the affinities or phylogenetic status of their specimens. The work will also be useful to those taxonomists who appreciate the value of plant anatomy in solving problems concerning the interrelationships of families. Taxonomists are frequently suspicious of the value of information secured with the aid of the microscope. This is readily understood while so little is known concerning the stability of anatomical characters under different environmental conditions. There can be little doubt, however, that many anatomical features are relatively conservative, and may safely be employed at least as confirmatory diagnostic characters. Since there thus seems to be good reason for supposing that attempts to correlate taxonomy and anatomy may lead to a more complete understanding of phylogeny, the compilation of *Forest Trees and Timbers* is an interesting experiment. It is difficult at this early stage to predict what will be the status of the work in time to come, but it may well be that it will stand out as a model to be imitated by others following similar lines of investigation. It is to be hoped that Dr Burtt Davy and Dr Chalk will continue the good work they have started and that their endeavours will be rewarded by a general appreciation of the value of their researches.

C. R. METCALFE.

Colloids in Agriculture. By C. E. MARSHALL. Pp. 184, 14 text-figures. Edward Arnold and Co. 5s. od. net.

This little text-book is interesting as a sign of the times. It offers concrete evidence of the great change in outlook of the soil sciences in the last thirty years. It emphasises in no uncertain manner the point of view that the fundamental chemical properties of the soil are those of its colloids.

It would be unfair, however, to convey the impression that it is mainly concerned with soil problems. It deals with the general principles of colloid chemistry and structure, giving, in illustration, examples drawn from soil, biological and dairy sciences. Its author may be congratulated on the lucidity with which these principles are explained and illustrated.

Apart from this, botanists will no doubt find the most interesting chapters to be those dealing with the colloidal materials present in living organisms. We may gently hint perhaps that there are vegetable proteins as well as those in animals, and that the biologist would undoubtedly welcome an extension of this chapter to include the colloidal system we call protoplasm. A field which closely concerns both physiologist and morphologist is the architecture

of biological colloids, particularly as shown by their X-ray structure. The summary of recent work in this field is both adequate and easily intelligible. There is much of interest also in the use of drought resistance and winter hardiness in crop plants in illustrating the problems of the water relations of gels.

The very large literature on the base exchanges in soils is too much for the ecologist to deal with. The summary of the present outlook given in this book should therefore prove extremely useful. Equally useful are the pages dealing with the development of soil types and with the colloidal properties of the soil organic matter, a field usually somewhat neglected, but of vital importance in plant succession. The whole makes an extremely useful text-book and one well worth consideration by those concerned with biological teaching.

W. H. PEARSALL.

Flore Laurentienne. By FRÈRE MARIE-VICTORIN, D.Sc. With 22 maps and 2800 drawings by Frère Alexandre. Montreal. 1935.
£1. 5s.

The publication of the *Flore Laurentienne* is an important event in the history of Canadian botany and a notable contribution to the science of taxonomy. As the author says, he has not attempted to write a complete flora of the Province of Quebec: he describes the vascular plants of the most densely populated and most accessible parts of the province, keeping before him the intention of enabling French Canadians to acquire a general knowledge, as exact as possible, of the native flora. The volume of over 900 pages includes a coloured map of part of the province, a concise history and bibliography of Laurentian botany, a general sketch of the Laurentian flora, a synopsis of plant groups, an analytical key by M. Jacques Rousseau, one of Prof. Marie-Victorin's colleagues in the University of Montreal, descriptions of 80 pteridophytes, 14 gymnosperms, 1251 dicotyledons, and 572 monocotyledons. There is also a statistical table of the flora, a glossary, and a full index. One wishes that more references to literature had been given.

Frère Marie-Victorin is much more than a leading authority on taxonomy; he has a profound love of nature and of his country—attributes familiar to readers of his delightful book of essays, *The Chopping Bee and other Laurentian Stories*. His literary gifts, his imagination and sense of humour are apparent in many passages of the admirable Introduction. His primary aim was to produce a Flora which should be living and human: in this he has been successful. He has added notes on ecology and on many other subjects which add greatly to the general interest of the purely descriptive sections of the book. The dedication is characteristic of the author: in the first paragraph he writes: "Je dédie ce livre à la jeunesse nouvelle de mon pays, et particulièrement aux dix mille jeunes gens et jeunes filles qui forment la pacifique armée des Cercles des Jeunes Naturalistes. Ce sera mon humble contribution à une œuvre pressante: le retour des intelligences aux bienfaisantes réalités de la Nature, au Livre admirable et trop souvent fermé, à cette Bible qui parle le même langage que l'autre, mais où si peu d'hommes savent lire les rythmes de beauté et les paroles de vie."

The Introduction is full of good things. After dealing with physiography and historical geology, climate, and the human factor, he gives a short description of regions, subregions and districts. The Arctic region is a narrow strip on the extreme north, tundra where mosses and lichens predominate, especially *Cladonia rangiferina*. The soil is permanently frozen to a considerable depth and thaws a foot or more below the surface during the short summer: the region is a vast expanse of peat, which makes a deep impression by its immensity, loneliness, uniformity and silence. Next comes the Hudson Bay region on a foundation of pre-Cambrian rocks, bounded on the south by the

isotherm 14° C. for the six warmest weeks of the year; an undulating and strongly glaciated country with numerous lakes, peaty swamps and *Sphagnum* bogs. The subarctic forests pass insensibly into the eastern coniferous forest of the Laurentian region, one of the largest forests in the world, separated by the prairies from the western group of conifers. This region is divided into subregions and districts. He speaks of the flora of the pre-Cambrian Shield as a young, aggressive flora capable of rapidly colonising wide spaces. The Gaspé district, a division of the Appalachian subregion, is described as, for the most part, a vast Nunatak never covered by ice, inhabited, as Fernald was the first to show, by survivals of an ancient interglacial flora, particularly on the Shickshock mountains.

The alluvial plain of the St Lawrence comprises several districts. Throughout many geological periods this, the oldest and most beautiful of rivers, has been a highway for migrating plants. On its waters floated seeds of Carboniferous pteridosperms and, after a lapse of many million years, seeds of cycadophytes, and again in a later age it had a share in the triumphal adventure of the flowering plants.

The flora of Anticosti and other islands in the Gulf is briefly described. Prof. Marie-Victorin points out that the state of equilibrium of the Laurentian flora implied by the description of regions and districts is an illusion, attributable not only to the fallibility of the individual observer, the "voyageur d'un jour qui s'arrête une heure", but especially to the short span of human knowledge.

In the latter part of the introductory sketch the author, admitting an evolutionary process in the past and in the present, discusses with his usual lucidity the data from the several regions in their bearing upon methods of evolution. In the section headed "Évolution à termes discontinus", he cites the genera *Senecio* and *Crataegus* as evidence of discontinuous evolution. Species of hawthorn in the Old World are few; in the New World the number is extraordinarily large. What is the nature of the "species" and what their stability? The great development of the genus in America is, it is suggested, a direct result of the disturbance of an ecological equilibrium. The Laurentian flora as known to the older botanists was very different from the flora we see to-day. European introductions are responsible for some of the most characteristic features of the countryside; the purple Loosestrife (*Lythrum salicaria*) and the Flowering Rush (*Butomus umbellatus*) have spread a purple and rose mantle along wide stretches bordering the St Lawrence. The spread of the Flowering Rush is particularly interesting.

Practically all species are illustrated: the drawings are good and their value is enhanced by the frequent addition of well-chosen details. It is impossible in a short review to do justice to Prof. Marie-Victorin's Flora; one can only leave it with confidence to the judgment of his fellow-botanists and warmly congratulate him and his co-workers on the completion of a great work characterised by breadth of vision, thoroughness of treatment and scholarly presentation.

The references to literature on Laurentian plants might with advantage have been more numerous, for example, the contributions of Prof. Fernald and of Prof. Marie-Victorin himself.

A. C. SEWARD.

Plant Viruses. By KENNETH M. SMITH. Pp. ix + 107, with 11 illustrations. Methuen and Co., Ltd. 1935. 3s. 6d. net.

The published work on plant viruses has in recent years assumed such proportions and is scattered in such widely different journals that it has become impossible for anyone not actively engaged in the work to keep pace with it. In this little book Dr Kenneth Smith, one of the foremost workers in the field,

has attempted a brief review, intended primarily for botanists and entomologists, of the present knowledge on these elusive pathogens.

All the main lines of virus work have been touched upon, and the ten chapters are full, perhaps rather too full, of information. It is perhaps inevitable in covering so great a field in so small a space that the book should in places tend to be a little disjointed and to lack sequence, but one feels that a little more criticism and guidance by the author might have been welcome, even at the expense of some information. Parts of chapter II, for example, seem too technical for such a book, for it is difficult to believe that the general reader will wish to construct an insect-proof glasshouse, or that the curiously undescribed Fig. 2 will help him even if he should. The other illustrations are, however, above criticism, being admirably clear and to the point.

In places there are tendencies to use virus and disease as if they were synonymous and to treat plant viruses as if they were all alike, but the meaning is usually clear and the reader has no difficulty in acquiring a considerable knowledge of the subject. Botanists, however, will probably be surprised in reading page 18 to find what wide applications tuber grafting appears to have in virus work.

The book has an index, and for those who desire still further information there is a bibliography of 11 books and 94 papers, selected to cover all the different types of virus work. At its modest price it is exceedingly good value.

F. C. BAWDON.

Plant Life: A Text-book of Botany. By D. B. SWINGLE. Pp. 441, 290 text-figures and a frontispiece. London: Chapman and Hall. 1935. 15s.

Prof. Swingle (of Montana State College) tells us in his preface that he has written this book to provide students with a botany course lasting one semester (which is a little longer than an English University term); it has twenty-nine chapters which might represent separate lectures.

The approach is "biological"—a synthesis of plant structure, plant physiology and plant life as crops or in the wild. Nearly the first half of the book deals with flowering plants; nearly the next half with the "types" of the plant kingdom, and finally there are a few chapters on general topics.

Plant Life is a book of character with much for a reader to like and dislike. The style is lucid, and, though never sensational, enlivened by enthusiasm and very occasionally rising to poetry, "...nuclei that will guide their destinies in the paths of their ancestors...". Most of the figures are new, and many are much better than the old text-book ones. It may also be said that the text is easy to understand: this is partly because difficulties are met by the simple method of omission. Where a difficult morphological conception is concerned, such as the foliar conception of floral organs or the rhizophore of *Selaginella* (dismissed simply as a root), there is very little loss. On the other hand the omission of considerable portions of plant physiology causes misgivings, and the numerous statements with which I disagree and would call errors are here serious because they affect a whole argument while in the morphological part their effect is pretty well confined to the sentence in which they occur.

The mechanism causing the ascent of sap is faced (the cohesion theory is outlined in a simplified form) but the whole subject of the part played by the cell wall in osmotic relations is omitted, i.e. turgor, suction pressure and plasmolysis. A student will probably gain a clear though rather inaccurate conception of the whole process of uptake of sap. I wonder if we must decide between teaching what we believe to be the truth at the risk of leaving a student's mind in a muddle and teaching something we think not quite right but which is understandable? An unpleasant dilemma. In justice to Prof.

Swingle, it must be pointed out that he avoids giving an impression of infallibility. A casual glance at the chapter on transport will cause shocks due to the wide use of the term osmosis to cover, apparently, all passage of substances through membranes: I suppose that physicists employ "osmosis" in this wide sense.

The part of the book dealing with systematics describes a number of plants which is astonishingly large, considering that this is only a half-year course, a wide survey evidently being preferred to the intensive study of a few types. Thus fourteen species or larger groups of fungi are discussed (besides myxomycetes, lichens, bacteria) and the algae similarly, but the bryophytes, ferns and gymnosperms are treated more economically and the systematics of the flowering plants is left out.

The final chapters on Mendelism, plant evolution, fossil plants, ecology and so on call for no special comment except that it is pleasant to see these interesting subjects given a considerable place in a brief course.

In an early chapter we are warned against teleology and in general the outlook is causal or at least does (just) avoid teleology, but the description of leaf fall, "A clever method is used by trees and shrubs for detaching their leaves", followed by a rider on the "purposes served by leaf fall", will cause violent reaction in those whose minds have become hyper-sensitised.

It should be said that while the great majority of plants considered are the same as those used in Britain, a few such as the sweet potato are not available, and such unfamiliar names as "buckeye", "basswood" and "squash" may prove slightly upsetting.

It may be concluded that any teacher of elementary botany might do well to study the methods of presentation in this book, and it might indeed be of direct use to school teachers, but its scope is too different from that of any syllabus I know for it to be recommended to British students.

T. M. HARRIS.

Gardening in East Africa: A Practical Handbook. Edited by A. J.

JEX-BLAKE, with a foreword by Sir ARTHUR W. HILL.

Pp. xv + 330, with six coloured plates. $8\frac{1}{2} \times 5\frac{1}{2}$ in. London: Longmans, Green and Co. 1934. 12s. 6d.

This pleasantly informative book is a compilation by members of the Kenya Horticultural Society and of the Kenya and Uganda Civil Services, edited by A. J. Jex-Blake. The Preface states that it attempts to meet a demand for a practical book dealing with gardening in Kenya, "where the climate is particularly favourable to the gardener". That this is no exaggerated claim for the climate is evident from the most cursory inspection of the contents of the book. A list of annuals which can be grown up-country in Kenya includes nearly all the most popular species of English gardens. Amongst herbaceous perennials many can be grown in the open, especially in moist and shady places, which are only greenhouse plants in England. But it is the list of climbers and of flowering trees and shrubs which arouses most envy in the stay-at-home breast. *Bignoniaceae*, *Bougainvilleas*, *Gloriosas*, *Ipomoeas*, *Landolphiias*, *Passifloras*, *Petrea volubilis*, *Tecomas*, *Thunbergias* all climb successfully, and amongst trees and shrubs we find *Cydonia japonica*, *Viburnum Tinus*, *Kerria japonica*, gorse, and roses cheek by jowl with species of *Allamanda*, *Bauhinia*, *Cassia*, *Dombeya*, *Rondeletia*, *Tibouchina*. Not quite everything will flourish: peonies and ordinary wallflowers will not flower, although Siberian wallflowers succeed; tulips are hardly satisfactory, and *Jackmanni* clematises refuse to climb. Most of our vegetables can be grown at high altitudes inland, though near the coast the gardener is advised not to waste his time on "peas, potatoes, seakale, rhubarb, parsnips and most of the cabbage family". All sorts of fruits, temperate,

sub-tropical and tropical can be grown at lower altitudes. Well-written chapters deal simply and straightforwardly with the practical side of gardening, preparing the soil, raising and propagating plants, controlling diseases, and the like. This book must surely have achieved what it set out to do.

It is a point of interest that so high a proportion of plants grown in England can also be grown successfully, at any rate at high altitudes, in Kenya. Most of the dicotyledonous herbs mentioned are natives of sub-tropical latitudes, presumably flowering in a day intermediate between the twelve-hour day of Kenya and the longer days of the English summer. It would be interesting to know if native English summer-flowering, and therefore long-day, species flower as freely in Kenya as do primroses, sweet violets, and daisies, all of which flower here in the twelve-hour days of early spring. A. R. CLAPHAM.

British Stem- and Leaf-Fungi (Coelomycetes). Vol. I. Sphaeropsidales.

By W. B. GROVE, M.A. Pp. xx + 488, with 31 text-figs.

8½ × 5½ in. Cambridge University Press. 1935. 21s. net.

Mr Grove's *British Rust Fungi* is well known and he has now increased the debt which all students of Fungi owe to him by undertaking to compile a systematic account of the British Fungi belonging to the important groups of the Sphaeropsidales and Melanconiales, which in some respects have been sadly neglected hitherto in this country. The present volume deals with the major part of the Sphaeropsidales and will be succeeded by a second volume which will complete the survey of the two groups. For many years the author has been an assiduous student of the micro-fungi, and his zeal and wealth of knowledge are fully displayed in this book. At long last Britain does not lag behind other countries in the possession of a treatise on these sections of the Fungi Imperfici. Its publication will be a great incentive to the further study of these fungi, many of which cause serious plant diseases.

In the Preface the author states that: "The account given of each species is in the main purely morphological; few pathological or cultural details are included, except very briefly. This is intentional. The two departments of Mycology, as experience many times has shown, are best treated by specialists, but working in happy conjunction." To the sentiment last expressed the reviewer subscribes most heartily. This sentiment, however, is not always honoured in the text. For instance, no one who has cultured *Phoma alternariacea* could think that it is "merely an early state of *Ascochyta Lycopersici*", as implied on p. 106.

Mr Grove is rightly critical of the delimitations of some of the genera included in the book. For example, he considers that it would be more logical to combine the genera *Ascochyta* and *Diplodina* instead of treating them as different entities. With further study of these fungi generic diagnoses are likely to be considerably altered, but it will need great courage for the systematists to break with tradition.

The references to literature are, in general, adequate, but the value of the book would have been further enhanced by the citation of additional recent papers on certain of the fungi such as *Phyllosticta Richardiae*, *Phoma Lavandulae*, *P. Lingam* and *Fuckelia conspicua*.

Some of the Sphaeropsidales are conidial stages of Ascomycetes, and the names of the latter, when known, are given in the text. It would have been advantageous if the names of these Ascomycetes had also been included in the Index.

At the end of the book twenty-four new species are described by the author, an indication of the fervour of his study. Altogether, the book is a very noteworthy production and a lasting testimony to the high achievement of one of the most veteran of mycologists.

F. T. BROOKS.

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THE EFFECT OF NOCTURNAL ILLUMINA-
TION BY DIFFERENT REGIONS OF THE
SPECTRUM ON THE SUBSEQUENT
OPENING OF FLOWER-BUDS

By NIGEL G. BALL

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(With 3 figures in the text)

INTRODUCTION

DURING the course of an investigation of the flowering of *Turnera ulmifolia* var. *elegans* it was found (Ball, 1933) that if the plants, or even cut shoots, are illuminated by artificial light during the night, the flowers which are due to open the following morning fail to do so. The corolla emerges from the calyx to the full extent, but it does not expand, and finally the whole perianth dies and becomes detached while the corolla is still in the closed condition. It was also found that the effect of one night's illumination is not confined to the buds which are due to open next morning, but extends to those which do not become mature until the second morning, even though these buds have been exposed to normal conditions during the intervening night. In such cases the flowers do not open fully and the petals have a crumpled appearance towards the tips.

In this plant the effect of nocturnal illumination on the opening of the flowers is closely correlated with a partial inhibition of the hydrolysis of starch in the petals. Under normal conditions the starch in the petals is rapidly hydrolysed before and during the opening of the flower-buds, but if the buds have been illuminated during the previous night the hydrolysis is to a large extent inhibited and considerable quantities of starch are retained in the petals. This inhibition is associated with a diminution in the diastatic activity of the illuminated petals.

In view of these results it appeared to be of interest to determine the effect of illuminating the buds during the night with different regions of the visible spectrum. In the preliminary experiments¹ a wooden box, open at the top but divided into two compartments by a central partition, was placed on a table in the laboratory directly below a 100-watt lamp. Several cut shoots of *Turnera ulmifolia* var. *elegans* were placed with their stems in water in each half of the box just before sunset. The two halves of the box were then covered with coloured filters and the lamp was switched on and kept burning until morning. Early next morning the shoots were removed and placed in front of a window and the effect of the treatment on the opening of the buds was observed. As controls, similar shoots were exposed overnight under a bell-jar to the full light of the lamp, while others were kept in the dark.

Standard light filters were not available at the time in Ceylon, so that improvised filters had to be used, the limits of transmission of the visible spectrum being determined by means of a spectrometer. Using a 100-watt lamp under a white shade at a distance of 1 m. from the buds it was found that the light which had passed through a flat glass dish containing a solution of ammoniacal copper sulphate, or even a plain copper sulphate solution, which absorbed the rays longer than about 620 m μ , had no inhibitory effect on the opening of the buds. On the other hand the same light, having passed through a sheet of ruby glass such as is used in a photographic dark-room lamp, was just as effective as the white light in preventing the subsequent unfolding of the corolla. This filter shut out the rays shorter than about 595 m μ . By placing on top of the ruby glass a glass dish containing a solution of the so-called "Light Green" microscopical stain, it was possible to cut out the rays below about 650 m μ and still to obtain the full inhibitory effect. In other words, under the conditions of the experiment, the violet to yellow regions of the spectrum were ineffective, while the full effect was obtained by the red rays alone. The efficiency of the red end of the spectrum is somewhat surprising, although of course these experiments are open to the objection that no account was taken of the energy transmitted through the different filters. In order that this objection might be removed, a new series of experiments was commenced. For this purpose standard light filters were obtained from Messrs Schott and

¹ An account of these experiments was read before Section K at the Leicester Meeting of the British Association for the Advancement of Science, 1933. *Rep. Brit. Ass.* 1933, p. 551.

Gen., Jena, and a Moll "Small Surface" thermopile from Messrs Kipp and Zonen, Delft. The filters were of glass 2 mm. thick and 8 cm. square.

METHODS

The experimental arrangements were as follows: A lamp house was constructed which could be suspended from the ceiling of the laboratory. In the bottom of the lamp house was a slide, with an aperture 7·1 cm. square, in which any one of the filters could be fitted. Above the aperture was a rectangular glass vessel through which water could be circulated, the depth of the water layer being about 5 cm. A 100-watt gas-filled lamp was fitted into the upper part of the lamp house, the filament of the bulb being 28 cm. above the lower surface of the filter. The arrangement of the lamp house is shown in Fig. 1.

Shoots of *Turnera ulmifolia* var. *elegans* with buds due to open the following morning were picked just before dusk; in dry weather the stems were cut under water. Four of these shoots were placed with their stems in water in a small glass vessel. At least four others were kept in another vessel as a control. In order to measure the energy value of the radiation, the thermopile was supported directly below the lamp house at a measured distance from the lower surface of the filter. As soon as darkness had set in, the lamp was switched on and the current generated in the thermopile was measured with a mirror galvanometer, readings being taken by means of a commutator on both sides of the zero. With a piece of plain glass in the position occupied by the filters and with the thermopile 1 m. below it, the sum of the deflections on both sides of the zero was 17·6 scale divisions. For the purpose of these experiments it was assumed that the galvanometer deflection was proportional to the energy of the radiation. After the deflection had been measured, the thermopile was removed and the vessel containing the shoots was supported below the lamp

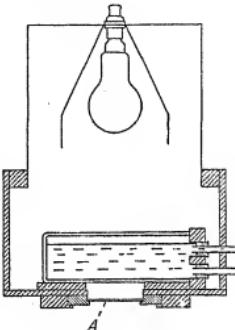


Fig. 1. Diagram showing the arrangement of lamp house and flower-buds. *A*, filter; *B*, conical reflector.

house, with the tips of the buds in the position which had been occupied by the sensitive surface of the thermopile. In order that the buds might be more evenly illuminated, the shoots were surrounded by a conical reflector of white paper, as shown in Fig. 1. The controls were kept in a dark corner of the room during the night. Next morning the experimental flowers and the controls were placed together on a bench in front of a window.

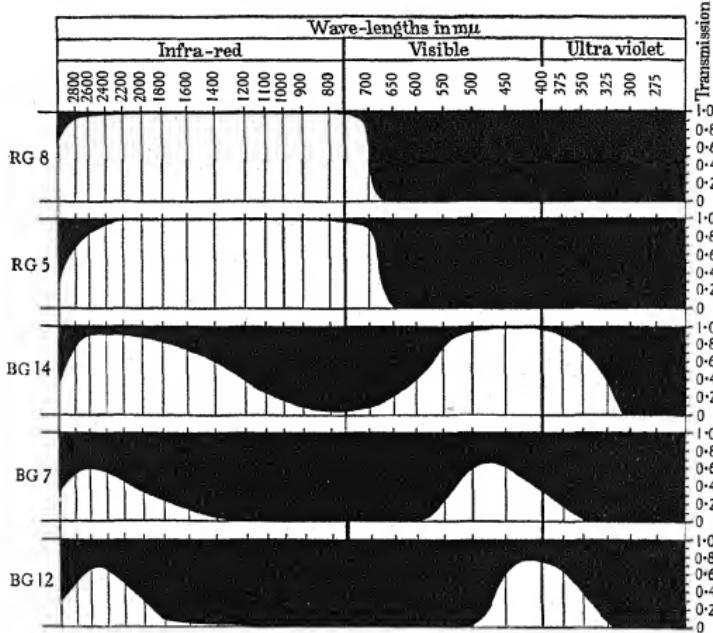


Fig. 2. Transmission curves of the filters used in these experiments, disregarding losses caused by reflection. Drawn from diagrams and data kindly supplied by the manufacturers, Messrs Schott and Gen., Jena.

In these experiments the five filters which were found to be most useful were RG 5, RG 8, BG 14, BG 7 and BG 12. A series of experiments was also carried out in which a piece of plain glass was substituted for the filter. The transmission of the different regions of the spectrum by the filters is shown in Fig. 2, which was drawn from diagrams and data supplied by the makers, Messrs Schott and Gen., Jena. The energy which was transmitted through the different filters varied very greatly. With the thermopile at a fixed distance below the lamp house, and with the water filter in position, the energy

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transmitted by each of the different filters, as indicated by the galvanometer deflection, was compared with that passed by the piece of plain glass. The results are given in Table I. Owing to fluctuations

TABLE I

Filter	Relative transmission of energy
Plain glass	100
RG 5	87.7 ± 0.89
RG 8	82.1 ± 1.00
BG 14	17.3 ± 0.24
BG 7	2.4 ± 0.07
BG 12	1.7 ± 0.06

in the voltage of the supply mains affecting the illumination, it was not possible to obtain any high degree of accuracy in these measurements. Because of the marked variation in the energy transmitted through the different filters, the distances below them at which the buds were placed in the different experiments varied considerably and ranged from 4.5 to 210 cm. Even this range was insufficient, and in some experiments it was necessary to change the source of light, the 100-watt lamp being replaced by a 400-watt or by a 500-watt bulb.

The quantitative estimation of the inhibiting effect of the illumination on the opening of the flower-buds is a matter of some difficulty. The method adopted, which appeared to be the only feasible one, was to measure the diameter of the corolla at intervals during the morning and to regard the maximum diameter as a standard of comparison. One cannot, of course, claim that the decrease in the maximum diameter bears a direct relation to the inhibiting effect of the light, but undoubtedly it does give a fairly reliable indication of the extent to which the flower has been affected. When inhibition was complete the diameter of the tip of the rolled-up corolla did not exceed 0.5 cm., while, when the opening had proceeded to the full extent, the diameter at the top was about 5 cm. The mean maximum diameter of 170 control flowers was 5.19 ± 0.042 cm. Between these two extremes various degrees of partial opening were obtained. The accuracy of the estimation of the extent to which inhibition had occurred was sometimes affected by variation in the maximum diameters of the four flowers used in each experiment. In the majority of cases these were reasonably consistent, but occasionally they differed to a considerable extent. In some cases these differences were obviously due to variation in the actual size of individual flowers. In others they may have been caused by inherent differences in

sensitivity or possibly by uneven illumination. Care was taken to arrange the shoots so that each of the buds was fully exposed to the light, but it was hardly possible to ensure absolute equality of illumination. In some experiments a difficulty was caused by the curling inwards of the edges of the petals, so that in extreme cases, instead of the corolla assuming the normal bell-shaped appearance, the petals became separated to form five divergent rays. Examples of this are seen in Pl. I, phot. 2, of an earlier paper (Ball, 1933). In the present series of experiments, which was carried out on cut shoots, extreme cases of this type of opening occurred only on very few occasions and such flowers were disregarded, as measurements of their diameters would have given a very false impression of the degree to which they had been affected by the light.

Owing to the fact that only one experiment could be carried out overnight, the whole series had to be spread over a considerable period. However, the variation in length of day and in temperature in Colombo is small and this plant produces flowers with equal profusion throughout the entire year. Such seasonal differences as were experienced did not appear to have any marked effect. An exception occurred during a short period when the room temperature each day exceeded 31° C., as lethal changes in the petals began to set in before anthesis was complete, and even the control flowers did not open fully. The results of these experiments and of a few others, in which for some undetermined reason the controls failed to open to the full extent, were rejected. In the successful experiments the temperature at the time when the flowers had reached their maximum opening varied from 25·8 to 30·3° C.

A factor which could not be controlled and which was undoubtedly responsible for a certain degree of inaccuracy in the energy measurements was the fluctuation in the voltage of the supply mains. With the thermopile in a fixed position below the lamp, variations up to about 10 per cent. in the galvanometer deflections were not uncommon. In order to make the determinations of the energy of the radiation in the different experiments as comparable as possible with one another, measurements were always made about the same time, that is, just after darkness had set in.

EXPERIMENTS ON *TURNERA ULMIFOLIA* L. VAR. *ELEGANS* URB.

The results of the series of experiments are recorded graphically in Fig. 3. In each experiment the mean of the maximum diameters of the four flowers is plotted against the energy of the illumination

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as represented by the deflection of the galvanometer when the thermopile was in the position occupied by the buds. Owing to the various sources of error mentioned above, a high degree of accuracy cannot be claimed for any individual experiment, but the plotted results with each filter lie fairly closely along definite curves. Where these curves are widely separated, the differences in the effects obtained with the corresponding filters are clearly significant.

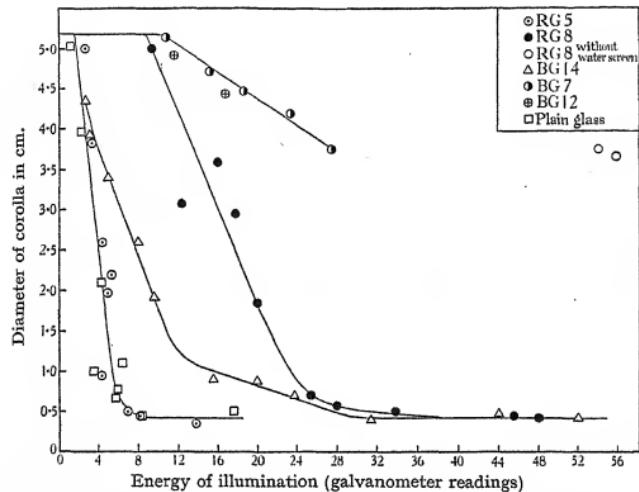


Fig. 3. Graphs showing the effects of nocturnal illumination through various filters on the subsequent opening of the flowers of *Turnera ulmifolia* var. *elegans*.

Using the filter RG 5, which cuts out wave-lengths below $650 \text{ m}\mu$ and passes almost completely those above $700 \text{ m}\mu$, there was no significant difference between the results and those which were obtained when the filter was replaced by a piece of plain glass. In other words, the light which has passed through a filter, which absorbs even more of the shorter wave-lengths than the ruby glass which is used in a photographic dark-room lamp, is just as effective in causing inhibition as white light, provided comparison is made with radiations of equal energy value. By using the filter RG 8 it was possible to determine the effect of the extreme red end of the visible spectrum. The transmission of this filter in the infra-red is similar to that of RG 5 and, as shown in Table I, the energy which it transmits is not

much less, but in the visible spectrum it cuts out completely the wave-lengths below 670 m μ and only transmits a small amount of those between 670 and 700 m μ . Although the difference between these two filters is very slight, there is a very marked difference in the results obtained with them. Complete inhibition was obtained with RG 8, but the energy of the illumination which was necessary was nearly four times as great as when RG 5 was used. Obviously, although the wave-lengths between 650 and 700 m μ are highly efficient in causing inhibition of anthesis, there is a considerable diminution in efficiency beyond 700 m μ .

The question as to whether the infra-red rays alone are effective was investigated in two ways. In the first, the filter RG 8 was used and the thermopile was placed 150 cm. below it. When the lamp was switched on, the deflection of the galvanometer was 8.4 scale divisions. As will be seen from the graph, this amount of energy with the filter RG 8 would have been insufficient to cause any inhibition. The water filter was then removed from the lamp house and the energy value of the radiation rose to 54.0 scale divisions. The buds were then placed in the position occupied by the thermopile and exposed to the light during the night. Next morning the flowers opened to about three-quarters their full extent. A repetition of the experiment on another night gave an almost identical result, and the results of the two experiments are indicated in Fig. 3. It will be seen from the graph that the same degree of inhibition which was obtained by raising the energy from 8.4 to 54 scale divisions would have been secured by increasing it by about one-eighth of this amount (8.4-14.0) if the water filter had been in position. The removal of the water filter, in addition to allowing a greater transmission to take place in the infra-red, would also have increased to some extent the energy in the visible portion of the spectrum owing to the absence of the reflection at the surfaces of the glass and water. It is reasonable to attribute a part, at any rate, of the slight inhibitory action which occurred in these two experiments to this increase and to conclude that little or no inhibition was due to the comparatively large amount of energy in the infra-red portion of the spectrum. This conclusion is confirmed by an experiment in which the buds were exposed to a source of infra-red radiation alone. Two heating units, each composed of thirty-seven turns of wire wound round strips of mica, 10 by 3 cm., were connected in parallel. On one side the wire was covered with mica and on the other it was exposed. The strips were supported horizontally between two clamps with the sides covered with bare

wire facing the floor. Eighteen shoots of *Turnera* were picked at dusk and placed in pairs in nine flasks of water. Two flasks were supported so that the buds were 35 cm. below the heater, two others so that this distance was 60 cm., while another two were placed on the floor with the buds 80 cm. below the heater. The shoots were arranged with just sufficient divergence from the vertical line to prevent the lower ones being shaded by those above. The six remaining shoots were kept as controls and were placed in another part of the room. The current was kept on from 6.45 p.m. until next morning at 7.50 a.m., and a resistance in the circuit was adjusted until the wire showed a very faint red glow in the dark. The current consumption was 4.2 amps. at 110 volts, and the resistance of the heater was 14 ohms when cold. Next morning the shoots were removed from under the heater and were placed with the controls in front of a window. All the flowers opened, and the course of opening was followed by measuring the diameters of the corollas at various times. There was no appreciable difference between the controls and the buds which had been 60 or 80 cm. from the heater. The buds which had been 35 cm. from the heater also showed no trace of inhibition and were actually about half an hour ahead of the others during the earlier stages of opening. These flowers also began to close about half an hour before the others. As a result of other observations, this earlier opening and closing can definitely be ascribed to the increased temperature to which the buds were subjected during the night. In view of these experiments and of the greatly diminished activity of the red rays beyond about 700 m μ , as was found in the experiments with the filter RG 8, there seems to be no doubt that the inhibiting action of the radiation from a lamp ceases at or about the limit of visibility at the red end of the spectrum.

With regard to the blue end of the spectrum, a series of observations was first made with the filter BG 14. As shown in Fig. 2, this transmits almost the whole of the visible spectrum up to about 500 m μ and a gradually diminishing proportion of the longer wave-lengths. From the graph in Fig. 3 it is clear that the inhibition was less than with RG 5, but more than with RG 8. Owing to the fact that the longer wave-lengths were not completely eliminated, it could not be determined whether the effect was due to these, or to the shorter ones which were transmitted at almost full strength. It is obvious, however, that the latter, even when a large proportion of the green and yellow rays is included, cannot have much effect. By using the filters BG 7 and BG 12 it was possible to eliminate the

wave-lengths beyond 600 and 500 m μ respectively. As will be seen from Table I, the proportion of the total energy transmitted by these filters is extremely small and, therefore, it is very difficult to raise the energy falling on the buds to a sufficient degree. However, by placing the buds only 4·5 cm. below the filter and bringing the 100-watt lamp closer to the water filter and finally by using a 500-watt lamp, it was possible to obtain results which were of value. These are shown in Fig. 3. With BG 7 there is very definite evidence of partial inhibition and, although it was not feasible to raise the energy value of the light beyond a certain limit, it is clear that the amount of inhibition increases slowly as the energy rises. With BG 12 the range within which it was possible to carry out experiments was even more limited, but with this filter also a slight inhibition was obtained. In addition to the fact that the maximum opening of the buds was less than that of the controls, the latter were definitely quicker in opening. So far as can be determined from the limited number of observations, there is no significant difference between the inhibitory effect of the radiation transmitted by BG 7 and that passed by BG 12, when compared at equal energy values, although, in addition to the violet and blue, the former passes a considerable proportion of the green and yellow rays. From these experiments it follows that, when the buds are exposed during the night to that portion of the visible spectrum which extends from the violet to the yellow, there is a very slight inhibitory effect on the subsequent opening of the flowers, but the effect is very much less than that caused by the shorter red rays of equal energy. A point to be noted in this connection is the difference which exists between the red and blue filters with regard to absorption in the infra-red region. Reference to Fig. 2 shows that the infra-red rays below about 1200 m μ are completely absorbed by the filters BG 7 and BG 12, while those of longer wave-lengths are transmitted. According to Jones (1929) a 2-cm. layer of water absorbs almost completely the radiation of wave-length greater than 1200 m μ , so that, with these filters and with the water filter in position, the energy which was measured was due entirely to wave-lengths of less than 600 m μ . On the other hand, with the red filters a considerable portion of the energy recorded by the thermopile must have been due to the infra-red rays below 1200 m μ which were able to pass through the water filter, but, as has been shown above, these rays have little or no effect in producing inhibition. If it had been feasible to eliminate these rays and to allow the radiation in the visible red to be transmitted in its entirety, there is no doubt that

the measurements of the energy necessary to cause inhibition with RG 5 would have been much less. As it is, a reference to the graphs shows that the red rays which were transmitted by RG 5 were about nine times as effective in causing inhibition as those which had passed through BG 7. If the infra-red rays had been cut out in the former case the difference would have been very much greater.

In a previous paper (Ball, 1933) it was shown that the effect of nocturnal illumination by white light on the opening of the flowers of this plant was correlated with a partial inhibition of the hydrolysis of starch in the corolla. In the present series of experiments with different regions of the spectrum the same was found to hold good. Whenever complete, or almost complete, inhibition of anthesis was obtained, there was much more starch in the affected petals than in those of the controls, but if the inhibition was only very slight the difference in the starch content was very much less and in some cases no difference could be detected.

EXPERIMENTS ON OTHER PLANTS

Before the thermopile and standard light filters were obtained, some tests had been made on cut shoots belonging to several other genera. Although the results are not based on measurements of the energy value of the radiation, they are of interest in connection with the question as to how far the results obtained with *Turnera ulmifolia* var. *elegans* are of general application. A 100-watt lamp was used at a distance of 1 m. from the buds, and, in addition to illuminating them with white light, they were also illuminated under the red and blue filters as used in the preliminary experiments on *Turnera*. To avoid repetition it may be stated at the outset that where a response to white light took place, similar results, both as regards inhibition of anthesis and inhibition of starch hydrolysis, were obtained with the red end of the spectrum, while under the blue filter the results were similar to those of experiments in which the buds were kept in the dark. In these experiments the illumination was not sufficient to secure the slight degree of inhibition with the shorter wave-lengths which was obtained with *Turnera* under very intense light. A point which should also be noted is that in every case where inhibition of anthesis occurred, the normal increase in size of the corolla took place during the night, the effect of the illumination being limited to the prevention of the divergence of the petals.

In the case of *T. ulmifolia* L., which differs in a number of minor characteristics from *T. ulmifolia* var. *elegans*, it was found that if

the buds were illuminated during the night the flowers did open, but opening was considerably delayed as compared with that of the controls. The corolla did not diverge to the full extent, and the edges of the petals towards the tips were curled inwards. The petals of such flowers contained large amounts of starch, whereas in the petals of the controls very little starch persisted after the flowers had opened.

Asystasia gangetica T. And., which differs from *Turnera* in the fact that its flowers remain fully open for from 1 to 3 days, also was affected when the buds were illuminated during the night before they were due to open. Normally, the lobes of the corolla begin to diverge about dawn and are fully expanded about 2 hours later. When the buds were illuminated during the night the flowers remained closed next morning, but the tips of the petals began to diverge slightly in the early afternoon. By evening such flowers were partly open and, if they were kept in the dark during that night, they had opened almost fully by the following morning, but the tips of the petals still remained slightly curled inwards. If, however, they were illuminated again during the second night, this inward curling of the petals was much more marked. On the third day, after darkness during the night, opening was complete. With regard to the effect on the starch content, it was found that, after being illuminated during the night before opening, the corolla contained a considerable amount of starch, whereas if the flower had been subjected to normal conditions the corolla had only slight traces near the base. In the former case the starch disappeared if a night's darkness followed the first night's illumination, but after illumination on the second night also there was still a considerable amount of starch, although less than on the previous day. If the buds were illuminated during the night but one before they were due to open, the divergence of the corolla lobes was delayed and did not commence until about noon. Such flowers contained much more starch than the controls.

In *Sesamum radiatum* Schum. and *Torenia crustacea* Cham. and Schl. there was partial inhibition of anthesis after the buds had been illuminated during the night before opening. In both species there was considerably more starch in the affected flowers than in the controls.

The experiments which were carried out on *Ipomoea Quamoclit* L. and *Ipomoea cairica* Sweet are of interest. In the former the opening of the flowers was completely inhibited if the buds were illuminated during the previous night; in the latter the flowers either remained closed or opened partially later in the day. In this species, if the

flowers which remained closed were kept in darkness during the next night, they opened to some extent on the following day, although normally by this time they would have completely faded. In this respect they behaved similarly to the flowers of *Hedera helix* as recorded by Sigmund (1929). With regard to the effects of the illumination on the starch content, it was found that the flowers of *Ipomoea Quamoclit*, whose opening had been inhibited by the light, had a marked amount of starch in the tips and edges of the lobes of the corolla, whereas in those which had been under normal conditions there was no starch except at the extreme tips of the petals. In *I. cairica* the flowers which had been illuminated had appreciable amounts of starch along the lines where the corolla had been folded in the bud, but in the normal ones only a few scattered starch grains could be detected in these regions. In both cases the remainder of the corolla was without starch.

In all these flowers there was a correlation between the inhibition of anthesis and a partial inhibition of the normal hydrolysis of starch, but an exception was found in the case of *Cassia occidentalis* L. In this species the flowers are ephemeral, and if the buds are exposed to light during the night they fail to open next morning. Starch is almost completely absent from the corolla, and even in buds, which were picked the evening before they were mature, it could only be found at the extreme base of the petals. Next morning there was still a trace of starch in this region, extending along the main veins to a distance of 1-2 mm. No difference could be detected between the starch content of flowers which had opened normally and of those whose opening had been inhibited.

Two species amongst those which were tested in Ceylon, eleven in all, gave completely negative results. The first, *Hibiscus rosa-sinensis* L., has flowers which open early in the morning and fade later in the day. When the buds were illuminated during the night before opening, or even during the previous night in addition, the flowers opened in the normal manner. The petals contained a considerable amount of starch, but no difference could be detected between the starch content of the illuminated flowers and of the controls. With *Commelina nudiflora* L. also negative results were obtained. In the case of *Cleome Burmanii* W. and A., the results were doubtful. Some of the experimental flowers appeared to be slightly affected, but others were normal.

DISCUSSION

From these experiments it appears that in a number of species belonging to different families the subsequent opening of the buds is wholly or partially inhibited if the buds have been exposed to light during the night before they are due to open. In other species this effect is entirely absent. During a visit to the British Isles in 1933 an opportunity was obtained of making some experiments on plants growing in a temperate climate and tests were made on the following species: *Campanula muralis*, *Cistus incana*, *Claytonia sibirica*, *Geranium anemonifolius*, *Hoheria* sp., *Iris* sp. and *Rosa spinosissima*. Buds due to open next morning were illuminated during the night while others were kept in the dark. In every case both the flowers which had been illuminated and the controls opened fully next morning. These experiments were carried out in the month of May and probably this explains the negative results. Sigmund (1929), who worked in October with *Hedera helix*, found that the opening of the buds was inhibited unless they had been kept in darkness for at least $5\frac{1}{2}$ hours before they were due to open. As he has pointed out, in this case he was dealing with a typical "short-day" plant. It seems probable, therefore, that an effect can only be looked for in plants which come into flower when the length of day does not greatly exceed 12 hours. In Ceylon such conditions obtain throughout the year, and the majority of plants which were tested responded to a greater or less extent when the buds were illuminated at night.

With regard to the efficacy of the various regions of the spectrum in causing inhibition of anthesis, the experiments which have been described show clearly that the maximum effect is exerted by the red rays about $650\text{--}700\text{ m}\mu$ in wave-length, and that there is a very considerable decrease in efficacy towards the blue end of the spectrum. This result forms a striking contrast to the well-known fact that phototropic stimulation is excited most strongly by the shorter wavelengths, the red rays having little or no effect. It is not surprising, therefore, that it should have been tacitly assumed by various writers that the same would hold good with regard to the effect of illumination on flower-buds. For example, Stoppel and Kniep (1911) made their observations on flowers, which were kept in the dark, by means of a red lamp such as is used in a photographic dark room. Schmucker (1928), in his work on the flowering of *Cereus grandiflorus*, employed the very weak light of a red lamp for a similar purpose, and Sigmund (1929) used red light for the examination of the inflorescences of

Hedera helix which were kept in the dark overnight. Although it is not suggested that the red light, in the short exposures which were used by these authors, is likely to have had any deleterious effect, it would appear that it did not afford any material advantage over white light of low intensity and that a comparatively strong blue light could have been used with absolute safety. Actually, in the present series of experiments it was found that the full inhibitory effect was exerted by a red light of which the visual intensity was extremely low, while a blue light which afforded quite a bright illumination had little or no effect.

Various experiments have been carried out on the effect of exposing plants for long periods to different regions of the visible spectrum. For example, Popp (1926) found that when plants were grown in daylight from which all wave-lengths shorter than $529 \text{ m}\mu$ were eliminated, the effects varied in different species, but all species, apart from the abundance of chlorophyll, had an etiolated appearance. There was also a considerable delay in the time of flowering and a reduction in the number of flowers produced. The presence of abundant chlorophyll is explained by the observations of Sayre (1928) that chlorophyll development in seedlings occurred in all regions of the visible spectrum, if sufficient energy were present, the red being most effective, followed by the green and blue. However, such experiments which deal with exposure day after day to definite regions of the spectrum do not appear to have any direct bearing on the present case, in which the effective illumination is confined to the period when the plant under ordinary conditions would be in darkness.

There is no doubt that in certain plants the regular alternation of light and darkness is necessary to bring about the full opening of the flowers. In *Turnera*, and in at least several other genera, one effect of nocturnal illumination, particularly by the red end of the spectrum, is the inhibition to a greater or less extent of the hydrolysis of starch in the petals. As has been shown previously (Ball, 1933), this inhibition may be associated with a decrease in the diastatic activity of the cell-sap. It would appear that there must also be an inhibition of other processes which tend to promote the divergence of the petals, at any rate in those flowers which contain little or no starch, but until further evidence has been obtained, any speculation as to the exact way in which the living cells are affected would be of little use.

SUMMARY

1. Flower-buds of *Turnera ulmifolia* var. *elegans*, which were due to open the following morning, were illuminated during the night by light which had passed through water and filters of coloured glass, the energy of the radiation being measured by means of a thermopile. The effect of the illumination on the subsequent opening of the flowers was observed.

2. When compared at equal energy values, the shorter red rays were found to be as effective as white light in causing inhibition of anthesis, but there was a decrease in efficiency with the longer red rays beyond 700 m μ . The infra-red rays produced little or no effect.

3. At equal energy values the yellow to violet region of the spectrum was much less effective than the red in preventing the opening of the flowers.

4. Inhibition of anthesis, either by white or coloured light, is closely correlated in this plant with a partial inhibition of the normal hydrolysis of starch in the petals.

5. Somewhat similar results were obtained with several other species, but in the case of *Cassia occidentalis* there was inhibition of anthesis although practically no starch occurs in the petals. In some other species no effect was observed when the buds had been illuminated during the night.

6. The efficacy of the longer wave-lengths in causing inhibition of anthesis, after the flower-buds have been illuminated by them during the night, forms a striking contrast to the well-known inefficiency of these rays in producing phototropic stimulation.

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ON THE SIGNIFICANCE OF MYCORRHIZA

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INTRODUCTION

IT is now generally recognised that the possession of mycorrhiza is not the characteristic only of a few somewhat abnormal families, but is a widespread phenomenon. Janse (1896), in Java, and Gallaud (1905) were among the first workers to indicate the large numbers of species involved. More recent are the observations of Asai (1934), in Japan, who recorded mycorrhiza in 82 per cent. of the 134 families investigated. McDougall and Glasgow (1929), in America, have recorded mycorrhiza on 28 species of Compositae; and Samuel (1926), in Australia, on 27 species of Leguminosae, 30 species of Gramineae, on Liliaceae, Ranunculaceae, Violaceae, Geraniaceae, Euphorbiaceae, Rosaceae, Plantaginaceae and Myrtaceae. A large number of species typically infected is given in the papers of Peyronel (1920, 1922, etc.). My own observations in eastern Australia have also shown that the phenomenon is widespread; Myrtaceae, Lauraceae, Saxifragaceae, Cunoniaceae, Sapindaceae, Rutaceae and Epacridaceae all possess members with mycorrhiza. Besides the above are families such as the Orchidaceae, Burmanniaceae, Ericaceae, Pyrolaceae, etc., which have long been recognised as mycotrophic, and also the large numbers of trees with their well-marked ectophytic mycorrhizas.

It was found that mycorrhiza fell naturally into several groups distinguished from one another by well-marked features. Ectophytic and endophytic forms were readily separated; in the former the main

mass of the mycelium remains on the surface of the roots and forms a mantle; in the latter most of the hyphae are in or between the cortical cells. Further observations have, however, shown that many intermediate forms also exist. The endophytic forms were further divided into those which had "pelotons", coiled masses of hyphae in the cells, as in the orchids, and those which formed arbuscules and vesicles. Arbuscules are comparable in general appearance with very much branched haustoria such as one finds in the Peronosporaceae, and vesicles are probably abortive sporangia. Peyronel has compared them with the sporangia of the Endogynaceae.

The researches of earlier workers, particularly Bernard, and then later Rayner, were responsible for the view generally held by most botanists, that there is a very specialised and balanced relationship between the fungus and the higher plant. This view was carried to the extreme by Bernard, who considered that for each species of orchid there was a distinct strain of fungus and that successful germination of the orchid seed was dependent on the presence of the appropriate strain of fungus.

PATHOLOGY

Extent of infection

Gradually the conception of mycorrhizal relationships has been extended. To Peyronel (1922, 1923, 1924) we owe the idea of "double infection" in some plants, the primary infection being by the arbuscule and vesicle former, the secondary by a *Rhizoctonia*. It is now recognised that tree mycorrhiza may be produced by numerous fungi, and the terms "true" and "pseudo" mycorrhiza have arisen. McDougall and Jacobs (1927) have recorded that with *Pinus mur-rayana* there are probably five fungi that form ectophytic and two endophytic associations. A similar diversity in the fungal element was found by Melin (1925) and Masui (1927). Perhaps the most striking lists are given by Peyronel (1921, 1922), who records *Betula alba* forming mycorrhiza with eight species of fungi, *Larix decidua* with eight species, *Corylus avellana* with nine, and *Fagus sylvatica* with thirteen.

Besides the difference in the specific nature of the fungi, considerable differences are also seen in the extent of infection. Apart from the known effect of external conditions which is discussed below, it seems essential that we recognise, too, the formation of what are best termed casual mycorrhiza, as in the Australian plant *Schell-*

hammera undulata (Liliaceae). The roots of this plant, a common herb in the sclerophyll forest of the east coast, are usually free from infection. However, a few plants examined showed infection of the lateral roots by a fungus of the *Rhizoctonia* type forming characteristic coiled masses of hyphae. This was not a seasonal feature because similar results were obtained many times throughout the year.

The influence of external conditions on endophytic mycorrhiza is as yet little understood. Reed and Frémont (1935) have shown that there are marked differences between the mycorrhiza of citrus trees grown under different manurial conditions. They found that "Roots growing in soils that had received no fertilisers during the preceding seven years had little power to resist invasion or to digest the intracellular mycelium. The mycelium seemed to grow as a pure parasite." In contrast to this they found that "Roots growing in soils which had annually received applications of cover crops and stable manure, appeared to develop a definite resistance to the invading fungus."

Samuel (1926) observed that the roots of oats growing in soil deficient in available manganese were heavily infected with an endophytic fungus. The roots of oats in normal soils were not so infected. During the Botanical Congress at Amsterdam, 1935, Dr Gerretsen of Gröningen showed exhibits in connection with the Grey Speck Disease of oats, which he believes is not a primary result of manganese deficiency but is the outcome of bacterial attack on the roots, following on a decrease of their resistance due to the deficiency of manganese. If this view is correct it suggests an explanation of the phenomenon observed by Samuel; the lowering of the resistance of the roots allows the fungus to become more prominent than is usually found.

In contrast to this increase in intensity of attack is the suppression of mycorrhizal formation by adverse environmental conditions. Rayner (1925) has shown that in *Calluna*, "the development of the endophyte is markedly inhibited by certain conditions of the rooting medium, and roots exposed to such conditions may appear to be uninfected". A suppression of the mycorrhiza is found, for example, in plants growing under very dry conditions.

Method of control

Whatever is the present nature of the relationship between the fungus and the higher plant, it appears that the association arose as a parasitic attack. From a study of the orchids it is possible to gain

some idea of how the fungal invader is controlled. Bernard (1911) and later Magrou (1924) showed that there is a toxic substance in the stem, leaves and tubers of the Ophrydeae which prevents the fungus entering these organs, and Fuchs and Ziegenspeck (1924) have shown the existence of enzymes which digest the fungal coils in *Neottia* and *Corallorrhiza*. They record glycogen, peptide and polypeptide splitting enzymes in the cell tissue. Working with *Orchis incarnata* I have been able to extract from the roots enzymatic substances which can cause disintegration of the hyphae, and have found that concentrated extracts from the roots of the orchid when added to the endophyte in culture, reduced the hyphae to a shrivelled oily mass in 3-4 days. Two methods of extraction were used. In the first the roots were shredded and then, after freezing and thawing, were subjected to considerable pressure. The expressed fluid was filtered and treated with excess alcohol to precipitate the enzymes. The precipitate was filtered off and redissolved in distilled water. The other method was far more difficult and was used for confirmatory tests only. Hand sections of the roots at least two cells thick were cut, and sap from cells at the actively digesting stage was removed by a micropipette controlled in a Janse-Peterfi micromanipulator. The sap from some thirty to forty cells was collected and placed in one drop on a cover-slip and allowed to dry. The residue was later dissolved in a much smaller volume of water. This method of obtaining the extract is attended with considerable difficulties but is the more conclusive. The effect of the extracts was observed by adding drops to the edge of the mycelium growing on cover-slips smeared with agar. It is hoped to publish fuller details of this work at a later date.

One is probably on safe ground in suggesting that a similar enzymatic action takes place during the digestion of arbuscules. So far inability to cultivate endophytes of this type on artificial media has prevented this from being tested. In *Eriostemon* (McLuckie and Burges, 1932) a more recent examination suggests that when the hyphae enter the cortical cells a modification of the hyphal tip follows, leading to softening and to extension of the wall at different places, thus rapidly giving numerous branches which form the arbuscules. The softening is continued and eventually the hyphal walls break down and allow the contents to be extruded by internal pressure. It is worth recording that during culture work with *Mucor racemosus* the hyphae in contact with contaminating bacteria in several plates became greatly altered; the hyphae branched freely and the ends of the branches swelled up and finally broke, the contents escaping on

to the agar. The branched hyphae closely resembled coarse arbuscules. Disintegration of the hyphae was attributed to bacterial secretions and may be considered comparable to the action of cell secretions on the arbuscules.

In the ectophytic mycorrhiza the problems differ somewhat. The fungi of most ectophytic mycorrhiza do not usually penetrate the cells of the roots. Instead there is a profuse development of hyphae resulting in the formation of a mantle surrounding the root. Melin's work (1925) reveals a possible explanation for the production of this mantle. He has shown that phosphatides excreted by the roots increase the growth of fungi associated with tree mycorrhiza. The amount of this increase varied considerably and appeared to depend to a large extent on the nature of the available nitrogen supply. The growth of *Mycelium radici sylvestris* was increased as much as 68 times by the addition of phosphatides from germinating *Pinus*, when ammonium chloride was used as the source of nitrogen; but with other sources of nitrogen the increase was much less, being of the order of 8-12 times. In the soil, excretion of phosphatides would lead to the formation of a zone around the root where local stimulation of the fungus would occur. Such a stimulation probably accounts in part for the formation of ectophytic mycorrhiza. The fungus responsible for the formation of the mantle will depend to some extent on the fungi present and on the conditions of the nutrient supply in the soil; changes in the soil conditions would lead to alteration in activity of the fungi and perhaps to change in the mycorrhiza.

Interpretation

In mycorrhizal studies much stress has been laid on the uniformity of fungal infection, and a great deal of significance attached to this. An attempt has been made above to show that this uniformity is more apparent than real; it is probably no more than the result of the uniform conditions in the soil leading to a uniform degree of disease.

Most phenomena associated with mycorrhiza can be interpreted as effects of parasitism. The enlargement of cortical cells, as in *Listera ovata*, is typical of hypertrophy as the result of parasitic invasion. The stunting and coraloid growth of tree mycorrhiza, the removal of starch and other food reserves from infected cells, are also indicative of parasitism. Enlargement and subsequent alteration in staining capacity of the cell nucleus has been interpreted by many workers as a sign of increased metabolic activities resulting from the

digestion of the fungal mycelium. However, this abnormal nuclear appearance can at times be found before digestion has begun, and similar changes occur in the nuclei of cells parasitised by rusts (Allen (1923) and Burges (1934)). Masni (1927), as the result of a large series of microchemical tests, concluded that *Abies firma* is definitely deprived of food materials by the mycorrhizal fungus *Cantharellus floccosus*, the relationship being one of parasitism by the fungus. Earlier the same author showed that during the months July and August, attacks of this fungus lead to the death of 60–90 per cent. of the lateral roots.

One seems justified in concluding that the mycorrhizal fungi, both ectophytic and endophytic, are potential parasites controlled by reactions of the host cells.

PHYSIOLOGY

Introduction and previous views

The second part of the problem of the significance of mycorrhiza concerns the fungi outside the roots. It seems certain that the fungi can assist some higher plants, as shown very clearly by the recent work of Rayner (1934) with conifers. Seedlings in plots to which the mycorrhizal fungi had been added showed a very marked difference in growth and seemed healthier plants in all respects when compared with plants lacking the mycorrhizal fungi. The extent to which plants avail themselves of such assistance varies. With saprophytic plants it is usually thought that the fungus supplies a considerable part, perhaps all, of the organic food material. So little is known at present of the general biology of saprophytic plants that it is difficult to evaluate the part played by mycorrhiza in this connection.

Fraser (1931) has examined two Australian species of *Lobelia* which are partly saprophytic and definitely mycorrhizal. The seeds of *Lobelia dentata*, one of the species studied, are extremely small, the average weight being slightly less than 0·01 mg., and the embryo is a small undifferentiated mass of cells. Germination frequently takes place several centimetres below the surface of the soil, and subterranean colourless seedlings of considerable size (2–3 cm.) were found. Such growth is mainly at the expense of food materials obtained outside the plant, since the food stored in seed weighing 0·01 mg. must be exceedingly small. Some pteridophyte gametophytes (*Psilotum*, *Tmesipteris* and some species of *Lycopodium*) have a comparable history; the microscopic spores with very small amounts

of stored food germinate in the soil and give rise to gametophytes about 0·5 cm. in length, frequently with abundant food reserves. These forms, too, are known to be mycotrophic. Saprophytic orchids must have a similar development. These plants, then, are able to accumulate much organic material despite their lack of chlorophyll. Since higher plants devoid of chlorophyll lack the ability to synthesise carbohydrates, one is justified in regarding the organic material in the soil as the source of elaborated carbon compounds, especially as saprophytic plants seem to be confined to soils relatively rich in humus.

Since mycorrhiza are constantly found in association with saprophytic plants a relationship between them has always seemed probable. Many hypotheses have been advanced to explain the physiological relations involved in mycorrhizal plants, and the more important views may be considered briefly.

Stahl (1900), as the result of detailed study of tree mycorrhiza, concluded that the fungus assisted the tree to absorb mineral salts and enable it to withstand the competition of the saprophytic fungi in the soil for these materials. Melin (1925), however, has shown that in pure cultures there was no significant difference between absorption by infected and uninfected roots. Nevertheless, it is possible that in soils rich in humus some of the mineral substances which become bound to the humus are liberated as the result of its breakdown by the fungal activity and become available for absorption.

The most widely held view in connection with endophytic forms is that material is transported from outside the plant along the hyphae, and is yielded up to the higher plant when the mycelium is disorganised. Various workers have laid emphasis on different substances detected in the cells; carbohydrate, fats, amino acids, proteins, have each at one time or another been thought to be the critical compounds involved. To me this idea seems untenable, and the oft-raised criticism that there are not sufficient connections between the external and internal mycelium justified. The fact that the endophyte can sometimes be replaced by a fungus which does not penetrate the roots but yet still produces the beneficial results, shows that penetration of the roots is not always an important factor (cf. Knudson, 1927; Freisleben, 1934).

About 1900, several workers showed that nitrifying bacteria were apparently absent from many heath soils, and it was suggested that fungi may take their place in the nitrogen cycle. Ternetz confirmed this observation and later (1904) isolated some five species of *Phoma*

from members of the Ericaceae and showed that they possessed a limited capacity to fix nitrogen. Despite the relative efficiency with which they worked, if one considered the amount of sugar used, the total amount of nitrogen fixed, even by the most efficient, was little more than five times the amount fixed by a species of *Penicillium*, while the endophyte from *Erica carnea* was inferior to this common mould. Nevertheless, Rayner (1922) claims that for *Calluna vulgaris* the endophyte fixes sufficient nitrogen for healthy seedling growth; in fact "the seedlings not supplied with nitrate were, on the average, healthier than the controls", to which potassium nitrate had been added. One cannot help feeling that this problem needs reinvestigating.

Orchid endophytes seem even less active. Wolff (1933) has examined a number of orchid endophytes and has shown that nitrogen is fixed, but his numerical values are very low. When the fungi responsible for ectophytic mycorrhiza on trees are examined, the evidence suggests that they lack any appreciable capacity to fix atmospheric nitrogen (Melin, 1925). In all the evidence so far advanced for nitrogen fixation by fungi, the amount fixed is so small when compared with experimental errors that the results must be accepted with caution.

The above views ascribe to the fungus a definite nutritional function, and it has frequently been the practice to consider seed germination in the Orchidaceae and Ericaceae as a somewhat distinct problem. The present view of germination in the Orchidaceae is discussed further on p. 126, and it is of interest to summarise the evidence for an obligate relationship in the Ericaceae at this point.

Rayner (1929), as the result of her pure culture experiments, concludes that, unless the seedlings of *Calluna vulgaris* were infected by the endophyte, "arrest of growth and the inevitable symptoms of malnutrition" followed. This result was in discordance with those of Christoph (1921) and Knudson (1929), who considered that infection was not essential. In a later paper Knudson (1933) records the result of his reinvestigation of the problem in the light of Rayner's criticisms of his previous technique, and has shown that *Calluna* seedlings can develop normally without the endophyte. Freisleben (1934) has reached a similar conclusion in respect to the genus *Vaccinium*.

The Australian family Epacridaceae is very closely allied to the Ericaceae, and the mycorrhiza is apparently of the same type. Recently McLennan (1935) has investigated seedling development under aseptic conditions in a species of *Epacris* and has shown that normal plants are formed without infection by the endophyte.

Present view

None of the above hypotheses seems adequate to fulfil the requirements of the data already obtained. Instead, I suggest that the essential features are:

- (a) The soil fungi break down the organic material in the soil into water soluble components.
- (b) These substances once in solution are available to the higher plant.
- (c) Some of these substances are absorbed directly by the roots of the higher plant.
- (d) Although some of the soil fungi are parasitic and are regularly found as endophytes in the roots, it does not mean that these fungi are necessarily the most important in the above decomposition.

Source of organic material.

The amount of organic material in the soil varies considerably in different habitats. Plant debris is continually being added, and at the same time breakdown of organic material is taking place; the net result determines the amount at any one time. Certain of the materials present in plant remains disappear rapidly, particularly those of low carbon-hydrogen ratio (sugars, starches, celluloses). Du Toit and Page (1932) have shown that there is an early loss of furfuroids, then a rapid decomposition of celluloses and finally a conversion of lignin and some cellulose into humic material. The breakdown of these substances is attributed to micro-organisms and a number of species have been cited (cf. Russell, 1932, pp. 318 *et seq.*). The action appears to be brought about by the excretion of enzymes which produce water-soluble breakdown products.

Fuchs and Ziegenspeck (1924) have shown that *Neottia* and *Corallorrhiza* contain enzymes which act on humus material in such a manner as to form soluble substances which can be absorbed through the cell membranes. "Die *Corallorrhiza* und *Neottia* enthalten Fermente, die auf die Humussubstanzen des Bodens wirken, sodass lösliche und durch die Membranen durchgehende Stoffe entstehen." In connection with the source of these enzymes they state that "Ob die Pflanze oder der Pilz der Ursprung dieser Körper ist, wissen wir nicht." It seems fairly certain that whether or not the *Neottia* or *Corallorrhiza* can produce the enzymes the fungus can, since it appears to be able to grow as a soil saprophyte and make use of the soil organic material. Some of my own observations support this view.

During an experimental investigation of *Orchis incarnata*, soil from the fen in which the plants were growing was sterilised by autoclaving and then inoculated with the endophyte. After 3 weeks, aqueous extracts of the substrate and culture were made, and tested for carbohydrates by Molisch's method. A similar test was made of tubes of sterilised soil not infected. Both showed the presence of carbohydrates, the former giving a strong reaction, the latter a weak one only. The inference is that this increase in carbohydrates in solution is the result of the breakdown of the soil organic matter by the fungus.

The fungi concerned in the breakdown under natural conditions need not necessarily be the mycorrhizal fungi. It seems probable that several other fungi unconnected with the mycorrhizal roots may be of importance. Such a view is expressed by Jahn (1934), who has developed a concept of a "peritrophic mycorrhiza". In such, the fungi appear to be common Mucorinae and Penicilliae which do not penetrate the root but function in the "rhizosphere".

Availability of organic material.

There appears to be no doubt that fungi in the soil can break down the insoluble matter and bring it into solution, but to estimate the extent to which it is then available to a higher plant is a difficult problem. It is best approached by considering Knudson's work on orchid germination.

It used to be thought that orchid seedlings were unable to germinate and grow on their own account. Bernard (1904) showed that if the endophytes were added to the cultures growth frequently followed. Knudson (1927) has extended this work and has shown that the important feature is not the penetration of the orchid by the endophyte, but the bringing of organic material into solution. For example, on a medium containing starch, the carbohydrate is unavailable to the orchid. If the fungus present causes hydrolysis of the starch, some of the resulting sugars, once in solution, are utilised by the orchid, which is then able to establish itself and later may become independent of this auxiliary food supply. If the organic material were supplied in solution, e.g. as fructose, no fungal agent was required. He further showed that other fungi such as *Phytophthora* could replace the endophyte and bring the organic material of the medium into solution. The fungi also caused a change in the hydrogen-ion concentration of the medium. This on its own did not lead to germination, yet it was shown to be a contributory factor.

Other workers have also shown the need for correct adjustment of the hydrogen-ion concentration of the medium.

This work of Knudson's shows clearly that, under culture conditions at least, the organic material once in solution is available to both fungus and the higher plant, and demonstrates also the ability of the orchid to assimilate simple water-soluble carbohydrates. In nature the carbohydrates are present in the humic fraction of the soil, and the importance of the fungi lies in their ability to break down this fraction.

Absorption of organic material.

An absorption of organic substances by the roots of higher plants is by no means just an aberrant feature of orchids. Knudson (1916) showed that *Zea*, *Pisum*, *Vicia*, etc. could absorb glucose, fructose, sucrose and maltose; Acton (1889) that *Acer*, *Quercus*, *Phaseolus*, *Euphorbia* could absorb glucose, sucrose and glycerin; and the absorption of malic, citric and tartaric acids was observed by Ravin (1913) for radish. Other workers have obtained similar results. Miller (1931, p. 503) has summarised the data for absorption of organic nitrogen by higher plants and concludes that "the experimental work would seem to show that green plants growing in a heavily manured soil in a field, or in a rich compost as frequently used in greenhouses, can absorb directly and utilise in their metabolism larger or smaller amounts of complex nitrogenous compounds".

Brigham (1917) examined in some detail the absorption of nitrogenous organic compounds by *Zea Mays*, and has shown that many substances are assimilated directly. Some particularly complex materials like peptone, alanine, linseed meal and cotton meal appeared to be more readily available after being acted on by *Bacillus subtilis*. This is, in effect, comparable to what I suggest occurs in saprophytic plants. Soil organisms of a fungal or a bacterial nature make the reserves of organic material in the soil available to the higher plant for direct absorption.

Falck (1923), as the result of a close study of the processes and organisms involved in the breakdown of humus in forest soils, concludes that the essential features in the mycorrhizal associations is the ability of the fungi to bring organic materials into solution and make them available to the higher plant.

Fuchs and Ziegenspeck (1924) are of the opinion that in *Neottia* and *Corallorrhiza* insufficient connections exist between the external

and internal mycelium for the transport of much material, and state that the products of humus decomposition are absorbed directly by the higher plant. Such I believe happens in the Australian plant *Dipodium punctatum* (cf. McLuckie, 1922) and suggest that the following, based on numerous field observations, indicates the mode of life of that species.

A common habitat for the orchid is a soil derived from sandstones and podsolised by the influence of fairly heavy rainfall (40–50 in. per year). The presence of large amounts of decaying leaves leads to the formation of a soil relatively rich in humus, and the warm climate aids rapid decomposition. Despite the rainfall the soil is frequently somewhat dry but is subjected to a temporary saturation following rain. Many of the plants growing in this habitat have structures which permit of water storage. Except at the time of flowering the *Dipodium* plant exists about 4–6 in. below the surface of the soil, as a short axis with a few large swollen roots with very well-defined velamen. Many of the cells of the cortex are filled with hyphae which undergo the usual changes associated with orchid endophytes. In plants dug up during the dry spells, the velamen is whitish and contains mostly air; during and immediately following rain, the velamen becomes greyish in colour and waterlogged. Micro-biological decomposition of the organic matter in the soil would lead to the formation of water-soluble substances which at the onset of rain would be washed down through the soil. It is this first water, relatively rich in organic matter, that would be absorbed by the velamen. Subsequent utilisation of the organic and inorganic materials then follows.

In holosaprophytes such as *Dipodium*, *Neottia* and *Corallorrhiza* there is no question of carbohydrate synthesis by chlorophyll. In many other orchids there is; and to evaluate the two sources of supply is difficult. Fuchs and Ziegenspeck (1924) have examined species of *Dactylorhiza* during the leafless period and have shown that there is an increase of 25 per cent. in the amount of carbohydrates and 42 per cent. increase in the amount of proteid nitrogen, which shows that appreciable amounts of organic material are obtained at the expense of the soil. In *Listera*, *Helleborine*, and probably in most orchids possessing a firm unswollen rhizome and abundant roots, I doubt if there is a significant addition of organic material from the soil. To these I would add the majority of mycotrophic plants which have well-developed leafy systems and abundant roots.

CONCLUSIONS

The presence of the fungus in a mycorrhizal association is to be regarded as an example of controlled parasitic attack and has no mutualistic significance. The fungi concerned are weak pathogens whose activity is curbed by the reactions of the host cells.

Distinct from this is the activity of soil organisms which break down the organic matter in the soil and render some of it water-soluble. Saprophytic plants make use of this breakdown and absorb some of the relatively complex organic materials directly through their roots, the amount varying with the degree of saprophytism.

The soil organisms responsible for the decomposition bear no necessary connection with the mycorrhizal fungi, but it is recognised that a fungus may conceivably act in both ways.

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THE EFFECT OF LIGHT ON THE ABSORPTION OF SALTS BY *ELODEA CANADENSIS*

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(With 2 figures in the text)

INTRODUCTION

LIGHT as a factor in the absorption of salts becomes particularly important in the case of water plants, where the absorbing cells in contact with the external solution are subject to changes in illumination. Hoagland and his co-workers (1923, 1926) have demonstrated an effect of light on the accumulation of certain inorganic ions by the cells of *Nitella*, but so far information is not available concerning the submerged aquatic angiosperms. The present paper gives the results of some preliminary work on this subject.

METHOD

Elodea canadensis was selected for experimental work as an example of a totally submerged aquatic. The material used was clean and free from obvious epiphytes or encrustation of calcium carbonate. In preparing the plants for use young healthy terminal shoots of uniform size (usually 4-5 cm. long) were picked. Each was washed by rubbing gently in a strong jet of water and subsequently in running tap water for about 24 hours. The shoots were then counted into four equal groups and each group was washed in several changes of the solution in which it was finally to be immersed. Then each batch of shoots was shaken free, as far as possible, from adhering solution and placed in a pyrex boiling tube of 80 c.c. capacity containing 40 c.c. of the experimental solution. It was necessary to take care that the shoots were distributed more or less uniformly in the tubes. When the four tubes were duly fitted up, the two which were to be illuminated were selected at random. The other two were darkened by wrapping them in several thicknesses of dark rubber sheeting.

The general arrangement of the apparatus used is shown in Fig. 1. Each boiling tube was fitted with a two-holed rubber bung with an inlet tube dipping almost to the bottom and an exit tube flush with the inner surface of the bung. The tubes were connected in series and

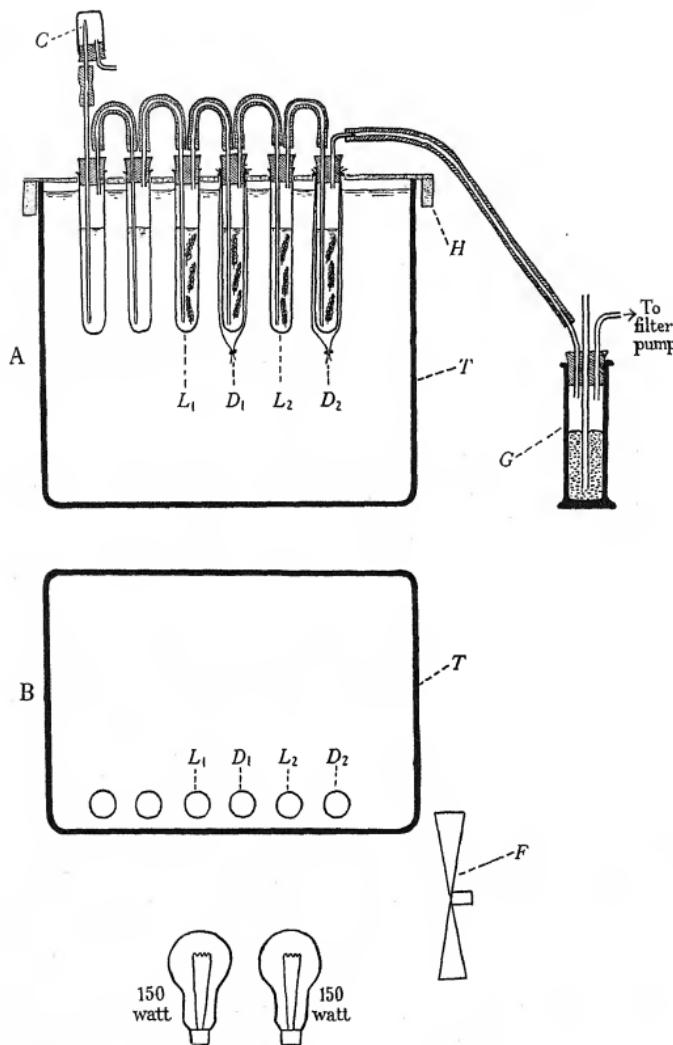


Fig. 1. Elevation (A) and plan (B) of the apparatus. *T*, glass tank; *H*, wooden tube-holder; *C*, fine capillary tube; *G*, mercury governor; *F*, electric fan; *L₁* and *L₂*, illuminated tubes; *D₁* and *D₂*, darkened tubes.

preceded by two others containing the same solution but without plant material. The object of these was to saturate the air stream with water vapour. Both stirring and aeration of the solutions were brought about by a continuous stream of air drawn through the apparatus during the course of an experiment. A perfectly constant current of air was maintained by inserting a mercury governor (Fig. 1, G) between the tubes and a filter pump and by making the resistance of the system largely dependent on a very fine capillary tube (Fig. 1, C), duly protected from dust, at the entrance to the system. The arrangement was so adjusted that air bubbled through the tubes at the rate of approximately 100 bubbles a minute. The volume of each bubble was about 0.02 c.c.

It might be objected that in so far as the air stream was concerned the four tubes L_1 , D_1 , L_2 and D_2 (see Fig. 1) were not under identical conditions. For instance, L_2 as compared with L_1 would tend to receive air containing more carbon dioxide picked up by bubbling through D_1 . That this objection has little weight is borne out by the fact that in all the experiments no consistent difference was observed either between L_1 and L_2 or between D_1 and D_2 .

The series of boiling tubes was disposed in a large rectangular tank of clear glass with the temperature controlled by an electric thermostat. A rapidly revolving stirrer kept the bath at a uniform temperature. It was so arranged that the illuminated tubes (L_1 and L_2) alternated with the darkened ones (D_1 and D_2).

Illumination was supplied by two 150-watt electric lamps placed in front of the tank, one directly opposite L_1 and the other opposite L_2 . The filament of each lamp was 15 cm. from the tank and 19 cm. from the centre of the nearest tube. To prevent undue heating of the side of the tank by these lamps an electric fan maintained a steady blast of air across the illuminated surface.

At the end of about 24 hours the tubes were detached from left to right and the solutions poured off for analysis. At the same time the shoots were collected, dried roughly with filter paper and weighed as a guide to the mass of tissue involved.

Most of the results relating to absorption were obtained for chloride on account of the rapidity with which the chlorine ion can be estimated by titration with silver nitrate. Some results refer to absorption of phosphate, and in these cases the volumetric method of Prescott (1914) was employed. For potassium analysis the cobaltinitrite method in the form described by Milne (1929) gave excellent results.

As a very rough guide to the amount of exosmosis into pure water the electrical conductivity was determined in certain cases.

In all cases pH determinations were made colorimetrically.

RESULTS

Table I gives the results of four experiments on the absorption of the chlorine ion from dilute solutions of potassium chloride by

TABLE I. *Absorption of chlorine ions from potassium chloride solutions.*

Twenty terminal shoots of Elodea canadensis in each tube with 40 c.c. solution. Period of experiment 23.5 hours. Temperature 22.5°C.

No. of exp.	Tube no.	Concentration of original solution <i>M</i>	Percentage absorbed	Comparison of means.			Fresh weight (final) in gm.
				Cl Value of <i>P</i>	Original pH	Final pH	
1	<i>L</i> ₁	0.005	7.8	0.015	6.0	7.0	2.55
	<i>L</i> ₂		6.7			7.0	2.43
	<i>D</i> ₁		0.0			7.8	2.14
	<i>D</i> ₂		1.0			7.8	2.18
2	<i>L</i> ₁	0.005	6.5	0.016	6.2	6.0	1.99
	<i>L</i> ₂		5.3			6.0	1.99
	<i>D</i> ₁		1.4			7.0	2.08
	<i>D</i> ₂		1.4			7.0	2.17
3	<i>L</i> ₁	0.005	3.5	0.036	6.2	7.0	Average 1.63
	<i>L</i> ₂		3.5			7.0	
	<i>D</i> ₁		1.4			7.8	
	<i>D</i> ₂		0.4			7.5	
4	<i>L</i> ₁	0.0025	15.0	0.014	6.2	5.8	3.02
	<i>L</i> ₂		15.1			6.0	2.68
	<i>D</i> ₁		1.9			6.2	2.77
	<i>D</i> ₂		4.7			6.4	2.37

TABLE II. *Absorption of potassium and chlorine ions from 0.01 M potassium chloride solutions. Twelve shoots of Elodea canadensis in each tube with 40 c.c. solution. Temperature 23.5°C.*

No. of exp.	Tube no.	Period of exp. hours	Percentage K absorbed	Comparison of means.		Comparison of means.		Fresh weight (final) in gm.
				Cl ab-sorbed	Value of <i>P</i>	Cl ab-sorbed	Value of <i>P</i>	
10	<i>L</i> ₁	22.0	11.6	0.015	6.4	<0.01	6.7	Average
	<i>L</i> ₂		9.2		6.0		6.6	2.45
	<i>D</i> ₁		-1.3		2.3		7.5	
	<i>D</i> ₂		0.0		2.5		7.3	
12	<i>L</i> ₁	23.5	12.9	<0.01	6.8	<0.01	6.4	2.47
	<i>L</i> ₂		11.2		7.3		6.5	2.39
	<i>D</i> ₁		2.7		2.5		7.7	2.42
	<i>D</i> ₂		2.7		2.5		7.7	2.47

9-2

TABLE III. Absorption of phosphate from 0.005 M potassium dihydrogen phosphate. Twelve shoots of *Elodea canadensis* in each tube with 40 c.c. solution

No. of exp.	Tube no.	Temp. °C.	Period of exp. hours	Per-cent- age PO ₄ ab- sorbed	Com- parison of means.			Fresh weight (final) in gm.
					Value of P	Initial pH	Final pH	
5	<i>L</i> ₁	21-23	21.7	17.0	<0.01	5.3	5.6	Average 3.33
	<i>L</i> ₂			16.6			5.6	
	<i>D</i> ₁			3.9			6.2	
	<i>D</i> ₂			3.9			6.1	
6	<i>L</i> ₁	24-28	22.5	14.3	<0.01	5.2	5.8	3.02
	<i>L</i> ₂			15.3			5.6	3.08
	<i>D</i> ₁			5.2			6.2	2.71
	<i>D</i> ₂			5.7			6.2	3.02
7	<i>L</i> ₁	23-25	23.0	16.1	<0.01	5.3	5.8	2.83
	<i>L</i> ₂			15.4			5.8	2.77
	<i>D</i> ₁			5.3			6.2	2.86
	<i>D</i> ₂			5.0			6.2	2.82

Elodea canadensis. The material used in Exps. 1-3 was grown in tubs and was somewhat impoverished. The shoots used in Exp. 4 came from a pond at Bracknell and were much more robust. For all later work very vigorous material from a pond with running water was employed. Table II contains results for the absorption of both ions of the salt. In the experiments summarised in Table III the absorption of phosphate from 0.005 M potassium dihydrogen phosphate was studied. In all cases (Tables I-III) it can be seen that very much more absorption occurred in the illuminated as compared with the darkened tubes.

For each experiment the mean absorption in *L*₁ and *L*₂ has been compared with that in *D*₁ and *D*₂ using "Student's" method (Fisher, 1928). If the limit of significance is taken as $P=0.05$, i.e. nineteen to one against the result being due to pure chance, the difference between absorption in the light and in the dark can in all cases be regarded as significant.

A curious and unexpected feature can be seen on examining the tables, namely, that in all cases the illuminated solutions were more acid than the unilluminated ones at the end of an experiment. It was thought to be of some interest to determine whether the same pH differences were to be observed in the case of *Elodea* shoots immersed in distilled water. This was found to be so, and exactly similar results have been obtained with *Ceratophyllum demersum* and *Potamogeton crispus*. In these experiments (Table IV) the electrical conductivity was also determined as perhaps affording a clue to the

amount of exosmosis of electrolytes. In all cases the conductivity of the solutions in the darkened tubes was very much greater than that

TABLE IV. *Twelve terminal shoots of Elodea canadensis in each tube with 40 c.c. distilled water. Period of experiment 23 hours. Temperature 23–24° C. Initial pH 6.4.*

No. of exp.	Tube no.	Final electrical conductivity (arbitrary units)*	Comparison of means. Value of P	Final pH	Fresh weight (final) in gm.
9	L_1	120	0.016	7.0	2.91
	L_2	129		6.9	2.88
	D_1	541		7.9	2.71
	D_2	660		8.0	2.99
II	L_1	140	0.012	7.0	2.37
	L_2	126		7.0	2.46
	D_1	471		8.0	2.34
	D_2	552		7.9	2.62

* In the same units a 0.001 N solution of potassium chloride has a conductivity of 798.

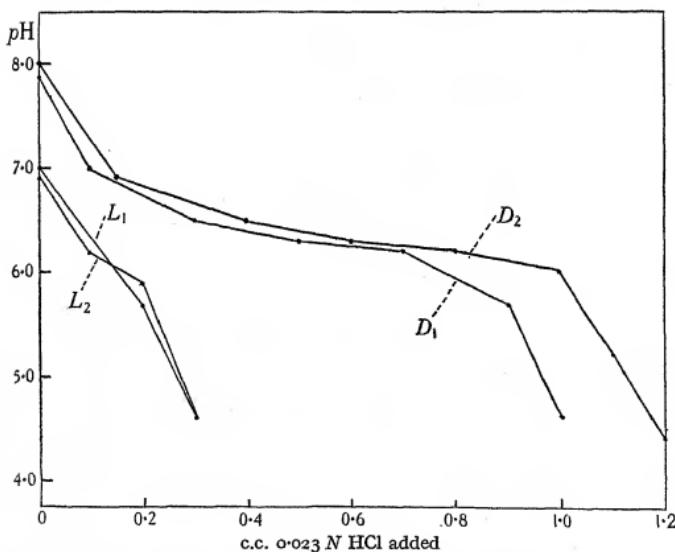


Fig. 2. Titration curves of solutions from L_1 , L_2 , D_1 and D_2 in Exp. 9. In each case 20 c.c. of solution were titrated.

of the illuminated solutions. The interpretation of these results is complicated by the fact that carbon dioxide is produced in the darkened

tubes and consumed in the illuminated ones. When, however, the conductivity results are considered in conjunction with the pH values there is a strong suggestion, at any rate in so far as cations other than the hydrogen ion are concerned, that light retards the net amount of outward diffusion.

The buffering of the solutions in these cases is of incidental interest. Fig. 2 shows the result of titrating with acid the solutions from L_1 , L_2 , D_1 and D_2 in Exp. 9 (Table IV). The solutions from the darkened tubes, as compared with those from the illuminated ones, are relatively highly buffered in the region pH 6-7. The titration curves for D_1 and D_2 are exactly like that given by a bicarbonate buffer mixture. This might possibly be confused with a phosphate buffer system, but chemical tests showed that no phosphate was present. It therefore appears probable that, when well aerated, *Elodea* immersed in water in the dark builds up in the external solution a relatively alkaline buffer mixture of bicarbonate of the form RHCO_3 .

Experiments were also carried out to test the effect of light on the absorption of chloride by *Ceratophyllum demersum* and *Myriophyllum* sp. In both cases results essentially similar to those for *Elodea canadensis* were obtained.

DISCUSSION

Numerous workers from the time of Tröndle (1910) have studied the effect of light on the permeability of plant cells, and the results in this field have recently been summarised by Lepeschkin (1930), who concludes that increase of permeability under the influence of illumination is a general property of protoplasm. He upholds the view that the effect of light is simply to alter the degree of permeability of the plasma membrane, and rejects the idea that light may affect an energy-consuming mechanism of absorption indirectly by way of photosynthesis. This latter view, which the present writer is inclined to support, is held by Hoagland *et al.* (1923, 1926) and the results obtained by him and his colleagues using *Nitella* support this theory. Work by Steward (1932, 1933) with potato disks, in which a close parallelism between salt absorption and respiration was observed, lends colour to the view that energy is involved in salt accumulation. There is little question that in certain cases illumination does increase the permeability of the plasma membrane. The results of Blackman and Paine (1918) on the exosmosis of electrolytes from

the pulvinus of *Mimosa pudica* find their simplest explanation in that theory, but it must be remembered that they were dealing with a tissue liable to spectacular changes in permeability. Jacques and Osterhout (1934) have recently reported an increased entry of potassium into the marine alga *Valonia* in illuminated as compared with darkened plants. This increase they believe to be associated with the rise in *pH* value just outside the protoplasm as a result of photosynthesis.

If we regard absorption as dependent both on the permeability of the plasma membrane and on the potential of active absorption, then it is clear that, when we measure net absorption, we cannot say how far this is the result of the intensity of active absorption and how far it is due to the limiting action of the plasma membrane. Thus in the experiments reported in this paper it is impossible to say whether light is influencing one or both of these factors.

Some experiments (see Table IV), in which outward diffusion of electrolytes into distilled water was considered, do suggest, in so far as cations other than hydrogen ions are concerned, that there is a decreased outward diffusion in the light. On the whole this is difficult to reconcile with the view that light increases the permeability of the plasma membrane. However, in this connection we must not lose sight of the possibility of the effect of carbon dioxide produced in respiration on the outward diffusion of cations. The entry of a cation into a cell depends, to some extent, on the anion with which it is associated. In the same way it may reasonably be supposed that the outward diffusion of a cation from a cell depends on its associated anion, consequently in the darkened cell where carbon dioxide is being produced and bicarbonate ions are formed the cations may possibly diffuse out more readily in association with the bicarbonate ions than in their absence.

It should be remembered that the temperature of the cells of illuminated green plants must be slightly above that of the surrounding medium, and this may in part account for the greater absorption in the light. However, this increase in temperature, amounting in all probably only to a fraction of a degree, would seem to be quite inadequate to account for the major part of the observed effect.

The more alkaline reaction acquired by the solutions in the unilluminated tubes as compared with the illuminated ones is of some interest. Theoretically the opposite result would seem much more probable, since the effect of photosynthesis in the undarkened

tubes is to remove carbon dioxide from the solution and so decrease the acidity, whereas respiration in the darkened tubes has the reverse effect. It is, however, easy to suggest a possible explanation of the observed result. If we suppose that the effect of light on the absorption and retention of ions is more marked in the case of cations than in the case of anions, then the observed differences might be expected to occur. The results for the absorption of the two ions from dilute potassium chloride solution (Table II) support this explanation. In these experiments light had a greater effect on the net absorption of the cation than on that of the anion.

If light affects salt absorption mainly by way of photosynthesis, as appears most likely at the moment, it follows that tissue devoid of chlorophyll should show little or no effect of light on the absorption of salts. Critical data on this point would be of value. Such evidence as exists is inconclusive. Steward (1932) with potato tissue could detect no effect of light on the absorption of bromine ion from potassium bromide, and a number of experiments by the present writer using excised roots of daffodil and disks of carrot root tissue agree, in general, with the results of Steward.

Again, if salt absorption in water plants is connected with carbon assimilation, increase of carbon dioxide in the air stream should increase absorption when this factor for assimilation is in relative minimum. It is hoped soon to apply this test to the theory.

SUMMARY

An apparatus is described for investigating the effect of light on the absorption of salts by water plants.

Results of experiments are reported which show, in the cases investigated, that light markedly increases the amount of salt absorption by *Elodea canadensis*.

In all cases the final hydrogen-ion concentration of the solutions containing illuminated *Elodea* was higher than that of the corresponding darkened solutions. It is suggested that this may be due to light affecting the absorption and retention of the cations more than that of the anions.

The question of the effect of light on salt absorption is briefly discussed.

My best thanks are due to Prof. T. M. Harris for his helpful criticism during the preparation of this paper for the press.

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ON THE DISTRIBUTION OF THE LIZARD
ORCHID (*HIMANTOGLOSSUM*
HIRCINUM KOCH)¹

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(With 5 figures in the text)

THE Lizard Orchid is, among the members of the British flora, of extraordinary interest, not only on account of its striking and unusual appearance but also because of its very sporadic occurrence. Very few native British plants are rare in the sense that they occur only in very small numbers and with great discontinuity of space and time, and of these, though *Epipogium* is perhaps the extreme case, the Lizard Orchid is certainly a most conspicuous example. Moreover, the latter plant has, during the present century, become more and more frequent over an increasingly large part of England, and thus affords the welcome spectacle of the increase, both in numbers and range, of a highly attractive member of the flora, a pleasing contrast to the normal effect of the passage of time on rare native plants.

This rapid recent spread is, however, only one chapter in the history of the plant in this country, and it was with the object of providing a comprehensive study of its distribution, both here and elsewhere since the earliest times, that this memoir was undertaken.

Enquiries made soon after the initiation of the work revealed that Mr W. H. Pearsall and Mr P. M. Hall were also engaged in collecting material for a publication with a somewhat more limited purpose. By their generosity a full exchange of notes was made and steps were taken to prevent overlapping in the presentation of results. Theirs have now appeared, in the paper already cited, and it is a great pleasure for me to have the opportunity of expressing here my indebtedness to this and my gratitude to its authors.

To collect anything approaching complete information about the distribution of any particular British plant is a lengthy and difficult process, since there is little finality about it and no obvious stage at which the absolute end of the task is reached. But apart from the

¹ In using this name I follow, without comment, Pearsall and Hall in *Report of the Botanical Exchange Club, Manchester, 1933*.

difficulty of tracing the records of rare plants, the records themselves vary greatly in authenticity, and it is necessary to decide which are of sufficient reliability to be used as a basis of study. In doing this it is clearly desirable to err on the right side and to exclude any about which there is reasonable room for doubt. For this reason the substantive list of records, which had to be compiled as a preliminary to further investigation, includes only those which are substantiated either by existent specimens or by circumstantial details coming from what may be regarded as reliable sources. Some records thus excluded are in all probability authentic, but these cannot be distinguished from others which are as certainly open to question and they have therefore been omitted.

The list in its final form contains 129 separate geographical records, and although the magnitude of this number will almost inevitably (in view of published statements) cause some surprise, it can be said with confidence that it is certainly less than a true statement of affairs, since not only are doubtful records excluded but also it obviously takes no account of occurrences of the plant which may have escaped all human notice. It would thus be idle to hope or maintain that the list is either complete or free from error, but every effort has been made to reduce the omissions and mistakes to a minimum, to trace known records to their sources, and to discover additional ones. An account of the actual means adopted to this end is incorporated with the original of the list, which it was not thought desirable to reproduce in full in these pages and which remains in my possession. The list was closed at the end of 1933 and does not include records of subsequent years.

The chief difficulty in the actual compilation of the list was to distinguish between separate localities and repeated occurrences in the same locality. In order to avoid confusion and ambiguity the word *record* is, in the following pages, used only in the former, geographical, sense, and the word *occurrence* is used with the latter, chronological, connotation.

CONTINENTAL RANGE OF THE LIZARD ORCHID

(Fig. I)

The Lizard Orchid has a wide range on the Continent of Europe and extends also into nearer Africa and Asia. Over Central, and especially southern Central, Europe its distribution is practically complete except for the high alpine parts of Switzerland and Austria,

but farther south it becomes conspicuously discontinuous. It is absent, for example, from much of Spain, including the lowlands, entirely from Portugal, from South Italy, and from parts of Greece. It is not known from the Balearic Islands and, although recorded, has never been confirmed from Corsica. Its range outside Europe is



Fig. 1. Map showing the approximate total range of the Lizard Orchid.

imperfectly known, and all that can usefully be said in the present state of our knowledge is that the plant has been noted in several parts of Morocco, Algeria and Tunis, from Cilicia in Asia Minor, and from farther east at Aintab.

It is also often quoted in literature as occurring on the island of Oesel in the eastern Baltic, the statement originating apparently in Ledebour's *Flora Rossica*. Such an isolated occurrence as this naturally called for some special investigation, and I therefore made

enquiries of the Botanical Department of the University of Tartu in Estonia. Thence I am informed that this record is but one of many similar errors based on the data of a collector named Luce. It may be added that similar records, with presumably the same origin, have been recorded for other British-occurring orchids.

From the point of view of the English distribution of the Lizard Orchid its northern limit on the Continent, which, in general terms, is along a line drawn from Boulogne to the mouth of the Danube, is of special interest. Taking country by country the actual limit is approximately as follows. In France it appears to be somewhere about the middle latitudes of the departments of Pas de Calais and Nord. In Belgium the plant is recorded very rarely from that portion of the country south of the latitude of Brussels. In Holland it was for a long time known only from a single locality in the limestone district of Limburg in the extreme south, but recently it has been recorded several times from the coastal sand-dune regions near the Hague, these localities forming a very marked outlier from its general continental range. In Germany the northern line is approximately that from Bonn to Dresden, and I have not heard of any records from Silesia. From Dresden the line continues more uncertainly, because of the increasing dearth of data, in the direction of Buda Pesth, containing only the south-western and southern parts of Czechoslovakia and the south-western half of Hungary. In Roumania the plant is recorded from the Dobrudja and from Transylvania, and there is apparently a large salient formed by the southern Carpathians, where it is absent. This northern limit is notably consonant with the land relief and seems to indicate that the plant is, at least in most of its continental latitudes, essentially one of medium elevations, avoiding both lowland and mountain areas. Its absence from the higher parts of the Alps and, farther south, from the less elevated parts of Spain and Italy, shows this feature well. Outside Europe the range is too incompletely known to permit of conclusions being drawn, but the repeated occurrence of the plant in the Atlas mountain ranges is significant.

The Lizard Orchid seems very rarely to be an abundant plant, and, for the most part, is rare and sporadic, but in places it is much more plentiful than elsewhere. This is true of France, western Germany, Switzerland and north Italy, and above all in that part of France between Paris and Bordeaux. In parts of this last-named region it has been described as occurring "in many thousands by the roadsides..." (Lacaïta, 1927). Northward from here it diminishes

rapidly and is, as I have said, apparently entirely absent from the extreme north-east corner of the country. Hence, the area in which it is specially plentiful on the Continent is not that nearest to England, and conversely in those parts nearest England it is either extremely rare or altogether absent.

DISTRIBUTION OF THE LIZARD ORCHID IN ENGLAND

(Figs. 2, 3, 4 and 5)

By the end of 1933 the Lizard Orchid had been recorded, in total, from 19 English Parliamentary counties and from 25 of the Watsonian vice-counties. In terms of the former it is known from Lincolnshire, Rutland, Cambridgeshire, Bedfordshire, Buckinghamshire, Oxfordshire, Gloucestershire, Somerset and Dorset, and from all counties to the east of these except Middlesex and London.

The actual western limit of the plant in this country is approximately a line joining, from north-east to south-west, Brigg, Grantham, Stamford, Cambridge, Dunstable, High Wycombe, Woodstock, Cheltenham, Devizes, Burnham, Salisbury and Dorchester.

The total number of separate localities (records) in England to the end of 1933 is 129. The total number of separate occurrences is 279, including four for which no accurate date is known. Of the records four need special comment. In three of them, namely, Burnham (North Somerset), Headington (Oxford) and Brigg—Caistor (North Lincolnshire), it has been suggested that the plant or plants were or are not of natural origin. As regards the first of these much has already been said (Stuart Thompson, 1924) and need not be repeated here, but the conclusion seems to be that, while the details of the locality arouse suspicion, there is no direct evidence of planting. A similar remark applies, as far as I am aware, to the second of them. The third case is rather different. This record is based upon an entry in a "Check-list of Lincolnshire plants" (Woodruffe-Peacock, 1909) in which it states "presumably planted". I have communicated with persons who know the circumstances of the discovery and they agree that the phrase used was based solely on the seeming unlikelihood of anything else, in the light of the contemporary knowledge of the plant's distribution. It must be remembered that at the time of the record, which was previous to 1909, the only record of the Lizard Orchid north of the Thames was that at Great Glemham in Suffolk in 1847, but in the light of subsequent information there seems no reason to suppose this Lincolnshire record was anything but a natural



Fig 2. Map showing the localities from which the Lizard Orchid was recorded up to the end of the year 1899. The dotted lines indicate the approximate boundaries of chalk soils. The large circle in the Dartford area includes 10 localities.



Fig. 3. Map showing the localities from which the Lizard Orchid was recorded during the years 1900-19 inclusive. The dotted lines indicate the approximate boundaries of chalk soils.



Fig. 4. Map showing the localities from which the Lizard Orchid was recorded during the years 1920-33 inclusive. The dotted lines indicate the approximate boundaries of chalk soils.



Fig. 5. Map showing the localities from which the Lizard Orchid has been recorded from the earliest dates to the end of 1933. The dotted lines indicate the approximate boundaries of chalk soils. The large circle in the Dartford area includes 11 localities.

one. I have therefore included it in my list and it remains of great importance because it was then, and still is, easily the most northerly record of the plant in England. Incidentally it is interesting, in view of it, to speculate whether, and if so when, the plant will reach the chalk wolds of the East Riding of Yorkshire.

The fourth record to be specially noted is an awkward and difficult one in several ways. It is from Bures, in the extreme north of Essex, within less than a mile, apparently, of the Suffolk border. Geographically it belongs to the latter region, whence there are several records, but politically it belongs to the county of Essex and is the first occurrence of the plant in the North Essex vice-county. Unfortunately, if there is an extant specimen I have failed to trace it, but the circumstantial details and authority are quite reliable.

The Channel Islands are not considered because they belong geographically to France and not to England.

Vice-county	Number of records	Date of 1st record	Date of 2nd record	Chronological order
E. Kent (15)	41	c. 1796	c. 1820	2
W. Kent (16)	18	1641	1809	1
E. Sussex (13)	10	1911	1914	10
Surrey (17)	9	1821	1878	3
E. Suffolk (25)	8	1847	1917	4
S. Hants. (11)	6	1925	1926	15
N. Hants. (12)	5	c. 1866	1884	6
W. Sussex (14)	4	1850	1907	5
S. Wilts. (8)	4	1907	1926	7
N. Wilts. (7)	2	1907	1913	7
N. Lincs. (54)	2	c. 1909	1929	8
E. Gloucs. (33)	2	1909	1917	9
Oxford (23)	2	1920	1933	12
Cambs. (29)	2	1921	1923	13
Berks. (22)	2	1921	1931	13
E. Norfolk (27)	2	1923	1932	14
Dorset (9)	2	1923	1933	14
N. Essex (19)	1	1919	—	11
N. Somerset (6)	1	1923	—	14
Bedford (30)	1	1930	—	16
Bucks. (24)	1	1931	—	17
Herts. (20)	1	1931	—	17
Rutland (55)	1	1931	—	17
S. Lincs. (53)	1	1931	—	17
Wight (10)	1	1933	—	18

Points to be noted specially are:

(1) The plant is recorded from vice-counties 6–17, 19, 20, 22–25, 27, 29, 30, 33, 53–55.

(2) The first record in East Sussex, a vice-county now third on the list, is as recent as 1911: in South Hampshire, sixth on the list, it is as recent as 1925.

(3) The long period between certain first and second records, i.e. 70 years in East Suffolk, 57 in West Sussex, 57 in Surrey. For reasons stated earlier the even longer space in West Kent is probably not an expression of the actual state of affairs.

(4) Four new county records in 1931.

Many of the details, and more especially the developmental details, of the English range of the Lizard Orchid are described a little later on, but the total statement is well summarised in the table on p. 151.

THE HISTORY OF THE DISTRIBUTION OF THE
LIZARD ORCHID IN ENGLAND

The history of the Lizard Orchid in England falls into three well-marked periods or phases. The first is the persistence of the plants in some or all of about 10 localities in the immediate neighbourhood of Dartford in Kent, from some unknown date prior to the middle of the seventeenth century till about the middle of the nineteenth century. The first of all English records was from this region in 1641, and thereabouts it seems to have persisted permanently for at least 200 years. It is true that I know of no specimens dated in the eighteenth century, but no doubt the plant is referred to in publications of that time, and I do not think the omission means that the plant died out for a while. Rather it seems to reflect the neglect of British field botany until the beginning of the collecting phase about 1800. This latter became intensified in the next few decades, and it is perhaps not without significance that the records multiply from this date to a maximum in the eighteen-forties. After the turn of the century the plant seems rapidly to have died out in these localities, quite possibly as a result of greedy collecting, and the last recorded occurrence there was in 1867.

Only once since then has the plant been found within the old Dartford area, and this was from what was apparently a new locality, in 1915.

The second period or phase, which was partly coincident with the first, is much less well defined and consists of 20 extremely sporadic records scattered over six vice-counties, including other parts of west Kent, between the years c. 1796 and 1899 inclusive. The vice-counties were, as will be seen from the previous table, East Kent, West Kent, Surrey, North Hampshire, East Suffolk and West Sussex. It is noteworthy that these 20 records comprise only twenty-three occurrences, that is to say in only three localities did the plant appear in more than one year. All three of these were in Kent.

It can thus be said that during this second phase nowhere outside the Dartford area did the plant occur anywhere but most transitorily. Indeed, towards the end of the period, after the Dartford plants finally died out, I have no occurrences at all in the following years, namely, 1869-74, 1876 and 1877, 1880-3, 1886, 1888-97. At this

time contemporary publications commonly referred to the plant as extinct or as verging on extinction.

With the new century the condition of affairs began to alter, and by the end of the first decade, 1909, the plant had been recorded from nine new localities, including four new vice-counties, namely, East Gloucester, North Wiltshire, South Wiltshire and North Lincolnshire. Furthermore, three old localities had shown fresh occurrences. This was the beginning of the third phase which is still continuing, but it was only a beginning, and since then the number both of localities and of occurrences has rapidly and steadily increased. The years 1910-19 (the latter year marks the end of the disturbed war period as well as the end of a decade) saw 19 new localities added, including two new vice-counties, North Essex and East Sussex, and seventeen fresh occurrences in four old localities.

But it is since 1919 that the spread of the plant has been most remarkable. In 1920 the plant appeared for the first time in Oxfordshire; in 1921 in Cambridgeshire and Berkshire; in 1923 in Dorset, East Norfolk and North Somerset; in 1925 in South Hampshire; in 1930 in Bedfordshire; in 1931 in Buckinghamshire, Hertfordshire, Rutland and South Lincolnshire; and in 1933 in the Isle of Wight. In all it was recorded during these years in 48 new localities (including 18 in East Kent) and as fresh occurrences in 16 old localities. The latter fact is interesting as showing that the plant was not only spreading but persisting in certain places.

If total records and occurrences are considered, then in 1900-9 there were 22 occurrences in 12 localities; in 1910-19 there were 38 occurrences in 23 localities; in 1920-9 there were 124 occurrences in 64 localities, while in the four subsequent years there were 49 occurrences in 34 localities, that is to say figures at the rate of 122 and 85 respectively for a whole decade. In addition to all these there are two localities and occurrences which cannot be dated accurately but which certainly are later than 1919.

The following table, which summarises the occurrences in the successive decades since 1800, emphasises several of the outstanding features of the above historical survey:

1800- 9	2	1870- 9	4
1810-19	1	1880- 9	3
1820- 9	5	1890- 9	2
1830- 9	4	1900- 9	22
1840- 9	10	1910-19	38
1850- 9	5	1920- 9	124
1860- 9	4	1930- 3	49

The figure 10 includes the peak of the Dartford specimens.

The rapid spread since 1900 deserves further study, and an easy method of analysis is to tabulate the total occurrences in the different years. The resulting figures are:

1900	1	1910	2	1920	9	1930	10
1901	1	1911	4	1921	15	1931	14
1902	1	1912	5	1922	10	1932	10
1903	1	1913	3	1923	12	1933	15
1904	1	1914	4	1924	19		
1905	2	1915	6	1925	14		
1906	3	1916	3	1926	17		
1907	4	1917	4	1927	12		
1908	3	1918	3	1928	8		
1909	5	1919	4	1929	8		

It will be seen even in this table that there is a certain rise and fall and that the increase is not evenly maintained, good years being eventually followed by poor years. This point is clearer, however, if only the new localities are considered, the figures of these being:

1900	—	1910	1	1920	4	1930	3
1901	—	1911	1	1921	8	1931	8
1902	—	1912	3	1922	3	1932	3
1903	—	1913	3	1923	5	1933	7
1904	—	1914	3	1924	5		
1905	1	1915	4	1925	8		
1906	—	1916	1	1926	8		
1907	3	1917	2	1927	2		
1908	1	1918	—	1928	3		
1909	4	1919	1	1929	2		

It will be seen from these tables that the highest number of occurrences in any one year is 19 in 1924, and the highest number of new localities in any one year is eight in 1921, 1925, 1926 and 1931.

FEATURES IN THE ENGLISH DISTRIBUTION OF THE LIZARD ORCHID

(1) Persistence in localities

The outstanding example of persistence in one locality is (excluding the old Dartford records) in one of the East Kent localities. Here the plant was first recorded in 1908, and it has been seen there every year since, a total of 26 seasons, and often in considerable numbers (see *infra*). Persistence of this order is, however, very rare, and no other instance compares with it. The next longest is 15 seasons, the length of time that a colony in West Sussex apparently lasted. Apparently because, I understand, that in late years the original plants have been gradually replaced by planted ones as they died out. Next in order is a persistence of 11 seasons in another East Kent locality. This case is particularly interesting because the period concerned was from 1898 to 1909, a time when the plant was at a low

ebb in England. The North Somerset locality also affords a persistence of 11 seasons, but this actually involves a period longer than that of the East Kent one just mentioned, because it ends only at the artificial restriction of my list to 1933 and the plants are still persisting in 1935. Then come two localities, one in Gloucester and one in Hampshire, where the plants have been recorded in nine successive seasons and in the latter of which it still occurs. Lastly there is a persistence of 5 years in an East Kent locality which I take to be the same locality as that in which a persistence of 11 years has already been mentioned, so that the plant has been recorded from this place in 16 seasons but not consecutively.

Apart from the above instances I have no records of persistence for more than three successive seasons, and this small figure only occurs three times, in Hampshire, Sussex and Wiltshire. Even two successive occurrences are far from common and have been noted only about 15 times. This is perhaps particularly noteworthy, since a find one year would be likely to cause a good search for the plant again the next season. On the other hand, failure to reappear must certainly sometimes be attributed to eradication by the finder.

Persistence may also be regarded from a rather different angle, namely, from that of total occurrences in localities rather than from that of successive appearances. Analysis on these lines shows that in roughly 60 per cent. of all localities the plant has been seen in one season only. In approximately 20 per cent. of localities it has been found in two seasons, and these are generally, but not necessarily, consecutive. In about 10 per cent. plants have been noted in three or four seasons, and beyond these numbers the percentages become negligible. The following table expresses this matter more fully:

Number of records (localities)	Number of occurrences (years or seasons)
79	1
23	2
9	3
6	4
1	5
1	6
2	9
1	11
1	16
1	17
1	26
4	?

(2) Length of time between successive occurrences

It has just been said that in about 60 per cent. of all localities the plant has been noted only in one season. The remaining 40 per cent. comprise, for the most part, localities in which occurrences have been in two or more successive years, but, in a small number, the later occurrences have been separated from the earlier ones by quite long periods of years.

The most remarkable of all these is one of the Surrey stations, where the plant was first found in 1821 and not again until 1927, a time gap of no less than 106 years. The next longest is in west Sussex, where the dates are 1850 and 1911, a gap of 61 years. Then come instances in East Kent (2) and North Wiltshire where the intervals are 38, 23 and 20 years respectively. There are also six localities in which the intervals between successive occurrences are between 10 and 20 years, and 17 in which they are more than 2 and less than 10 years. It is perhaps worth mentioning that among these last the interval of 6 years is specially common, occurring in no less than eight instances.

In total my records show some 40 localities in which, since 1850, there have been more than one occurrence. These comprise 139 occurrences in all, separated, in the different localities, by time intervals of 1 year or more. In 103 of these the plant has been found in actually successive seasons, that is to say with approximately 12 months between appearances, but in 36 the intervals are longer. Among these the interval of 2 years occurs seven times, of 3 years five times, of 4 once, of 5 once, of 6 eight times, of 8 three times. In the remainder the intervals are 9, 10, 11, 12, 14, 16, 20, 23, 38, 61 and 106 years. In one sense these varied time intervals are expressions only of a purely human factor, namely, the success or failure of search for the plant. A definite record proves the presence of them, but it cannot be said that the absence of a record proves the reverse. The plant may have occurred once or more often unobserved. The extent of this cannot of course be gauged. Moreover, the question of identical localisation is particularly important here. A time interval is clearly more significant if, at the two extremities of it, the plant or plants occur in exactly the same place. In such circumstances the suggestion that they are direct descendants of one another is much stronger than would otherwise be the case, but this information is rarely if ever available. Again, if the plant is constantly appearing afresh by

the processes of dispersal there is some chance, however slight, that it may be found more than once in practically the same place.

At the same time the number of what appear to be successive occurrences in the same locality is such as to suggest that there is something more than these possibilities behind them. This is true especially of the shorter intervals, which certainly suggest the persistence of the plant unnoticed, and presumably below ground, for at least some seasons. In this respect the longer intervals are not so striking. In the Surrey locality already alluded to, for example, the actual station is a place constantly visited by both botanists and others, and it is very difficult to believe that the plant has occurred here, even if rarely, without being noticed in the 106 years. If it has not so appeared it is equally difficult to believe that it has nevertheless persisted there in some unrecognisable form.

Similarly with the other long intervals, but with the shorter ones the possibility that the time gap is that required for the full development of seedlings of the earlier plants has to be taken into account. It will be seen later that there is some evidence that the development of the Lizard Orchid from seed to flower does occupy several seasons and that this may be the explanation of the shorter time intervals. It was in this connection that the rather common interval of about 6 years was emphasised earlier.

(3) *Number of plants*

Unfortunately the number of individual plants found is not very often stated, but the general sparsity of the Lizard Orchid is well illustrated by the fact that among the 70 occurrences in which numbers are recorded, in 46 there were only single plants. In five cases two plants were reported, in five cases also there were three, and in one there were four. On two occasions about a dozen were noted, and this kind of figure may be regarded as a general maximum. In three localities, however, the plant has appeared in much greater abundance. The first of these was in 1850 in West Sussex, when it was described as present "in great numbers". The second is in one of the East Kent localities where the plant is persistent. Here, over 7 years for which I have figures, the average number has exceeded 20. Lastly there is the North Somerset locality, where, for several years, the number of individuals has ranged between 30 and 100. The latter figure is the largest ever recorded in this country.

The records of single individuals are more significant. These are very numerous, and this seems to indicate that the plants have arisen

from a wide scattering of disseminules and are completely fresh occurrences. If they were derived from closely neighbouring plants and particularly as seedlings they would, I think, tend to occur more in small aggregates. The fact that in many localities only one plant has ever been found is perhaps additional support for this view.

(4) *Habitat*

Over much of its range the Lizard Orchid is pre-eminently a plant of the chalk, and this is particularly true of England, as will be seen from the maps in Figs. 2, 3, 4 and 5, on which are given the approximate limits of the chalk soils in this country. These maps, indeed, show that of the 129 localities from which the plant has been recorded, over 80 per cent. (one or two are so much on the boundary as to be uncertain) are on the chalk, and this soil preference is confirmed by such meagre habitat notes as are available. These are unfortunately few, but among them references to chalk downs and other chalk habitats predominate strongly.

Next to the chalk the plant has been most often recorded from soils of the Lower Oolite, a formation in which limestones are common. In fact, with one exception, all the localities west of the chalk are in this zone and generally on limestone, although in one case at least the plant was found on Forest Marble.

The one exception just mentioned is in north Somerset, where the habitat is on sand near the coast. It may be remembered in connection with this that the Lizard Orchid is found, though rarely, in similar situations in Holland, and doubtless also elsewhere abroad.

There remain about a dozen English localities to be accounted for, and it is noteworthy that nine of them are east of the chalk in Essex, Suffolk and Norfolk, that is to say, on deposits of Eocene, Pliocene or more recent age. In one case the habitat was a gravelly bank above a river, in another the retaining wall of a river, in one dry Coralline Crag, and in three it was on glacial deposits. For the rest there is no information.

Besides all these there are three localities in south Kent and north-east Sussex, of which one and perhaps two are on the Wealden and the other on alluvium. There is also a single record from the Oligocene soils of the Isle of Wight.

As regards the more detailed features of the habitat the extant information is still more meagre. Chalk downs have already been mentioned as predominating, but it is surprising to find that next to these the situation most often recorded is by the sides of roads or

paths. At least six such are definitely mentioned, and there is reason to believe that there have been other similar occurrences. It will be remembered, too, that a like remark has been quoted in connection with the plant in France. As to the rest, the plant has once been recorded as growing in a Martello tower, in a wood, and a retaining wall has already been cited.

It may be added here that references to ripe seed are not uncommon, and there are one or two mentions of presumed seedlings.

THE BIOLOGY AND CULTIVATION OF THE LIZARD ORCHID

The notes associated with the records of the Lizard Orchid in its natural habitats afford practically no information about its biology, and for this it is necessary to turn to other sources and particularly to the experiences of those who have attempted to rear and grow the plant in cultivation (Correvon, 1898; Mütze, 1897; Wooster, 1935).

Like many other of our native orchids a complete plant of the Lizard Orchid consists of an inflorescence, an inflorescence stem, a rosette of a few radical leaves, and a root system comprising a few short thick roots and two ovoid tubers. The last named belong to two successive active seasons, the older being in process of exhaustion while the younger is being formed. The tubers are organs of food storage and meet the heavy strain of flowering. Whether the ability to flower depends entirely or chiefly on the amount of reserve food available in the tuber has not as far as I know been established, but it is at least probable that there is a relation between the two. If so it would seem that flowering is very much more likely to occur the year after a season in which the conditions for food manufacture have been especially good. Conversely it might be expected that a succession of suboptimal seasons might result in prolonged failure to flower. On the same basis it is unlikely that the climatic conditions in any season would appreciably affect the flowering of the plant in that season. At any rate it is fairly safe to assume that the uncertainty of flowering in the Lizard Orchid, at least in England, is due to some such relation between external conditions and metabolism.

It is important to realise that the Lizard Orchid, and other similar members of the same family, are, in effect though not in fact, annuals. This is because each year's newly formed tubers gives rise, in the following season, to the whole of the functional plant. The old and new tubers remain in organic contact, but by the beginning of a new season the old tuber has normally become completely exhausted and

contributes nothing to the year's growth. Each season's plant arises from a tuber which is a daughter tuber of the previous plant and thus is itself always a daughter plant. It is significant that in these circumstances the persistence of the plant from year to year depends, not upon the general robustness or stamina of any one flowering individual, but upon the successful yearly formation of a new tuber. This may well be controlled by considerations or factors quite different from those which control the successive appearance of a true perennial plant in which the same plant body lasts for many seasons. There can, for instance, be little in the nature of an accumulated reserve power, and lack of this must certainly lead to an increased likelihood of irregularity of appearance. It is difficult to resist the impression, too, that this particular type of perennation is especially likely to reflect, in irregularities of occurrence, the periodic suboptimal conditions which the plant is likely to meet on the limits of its range, and that it therefore affords at least a partial explanation of the main features of its distribution in England. It also follows that the destruction and death of a daughter tuber results in the termination of the individual lineage which gave rise to it.

The experience of growers of the orchid is in good accord with what has just been said. The tubers are frequently quoted in the lists of continental seedsmen, and plants from such sources are not uncommonly to be found in English gardens. From the general experience of those who have so grown them, three main statements emerge. The first is that the plants almost invariably dwindle and die out after a very few years. The second is that they often die after flowering once, and thus seem, more often than not, to be mono-spermic. The third statement is that the germination of seed, which is quite commonly produced, is very rarely, if ever, successful. In addition, there are among growers of the plant repeated references to the harmful effects of frost, especially on tubers from abroad.

Besides being raised from imported tubers the orchid is not infrequently grown from tubers or whole plants transferred to gardens from natural English habitats. When this is done there is little difficulty in making the plants do well for a time, but after a while these also tend to show the dwindling already mentioned.

A recent example of such transplantation seems to have been especially successful (Wooster, 1935). In this case a plant was in 1931 lifted when in early bud from its natural position and placed in a garden. It flowered that year though rather late, but failed to do so in 1932 and 1933, although the vegetative parts remained

strong. Similarly in 1934 it produced no flower, but five seedlings, presumably derived from the 1931 flowers, some of which ripened, appeared. Following the three barren years the plant flowered once more, exceptionally well, in 1935, the spike lasting for 5 weeks and bearing over 150 flowers, about 25 per cent. of which formed ripe seed capsules. At the present time the five seedlings survive strongly, and it will be interesting to see whether, and if so when, they eventually flower, as this will furnish authentic information as to the time which may elapse between seed production and flower production—that is to say between successive seed generations.

On one occasion at least imported tubers were deliberately grown in soil taken from an English locality in which the plant had once been recorded, but this did not prevent the plants from dying out in a year or two.

Besides being cultivated in gardens imported tubers have, apparently on several occasions, been planted in natural English habitats in order to reinforce or replace native colonies which showed signs of dying out. This seems to have had the desired effect, at any rate for a time.

I have also heard of a colony of tubers which was planted in a wild spot in Kent but not in one of the orchid's known localities. These plants also survived for a few years, but I do not know their ultimate fate.

Reference to literature (Burgeff, 1909; Fuchs and Ziegenspeck, 1927), to which I was directed by the courtesy of Prof. Burgeff of Warzburg, shows that the Lizard Orchid, like so many of its family, possesses mycorrhiza. To what extent the features noted above in the cultivation of the plant and discernible also in wild individuals are to be attributed to this cannot easily be estimated, but it is worth noting that if the tubers contain a fungus, and that this requires to be reinforced after a time by further infection from the soil, the failure of this process in soils destitute of the appropriate fungus would be very likely to result in the dwindling and monospermic effects which have been so frequently noticed. It may at least be surmised that the mycorrhizal nature of the plant is yet another of the factors which contribute to the peculiarities observable in its distribution.

DISCUSSION

It has been shown that certain features in the English distribution of the Lizard Orchid, such as its sporadic and transitory occurrence, may be attributed to its method of perennation and to the fact that

it possesses mycorrhiza, but these can hardly be the direct explanation of the most outstanding aspect of its history in this country, namely, its sudden increase from what was in 1900 an almost extinct state.

Bearing in mind, on the other hand, that the occurrence of a plant in any area is determined primarily by the climatic conditions and secondarily by the edaphic conditions there prevailing, any attempt to explain the vicissitudes of the orchid in England must embody the possibility of changes in one or both of these over the appropriate period, that is to say from c. 1850 to 1933.

The question of edaphic change, that is changes in soil condition not directly associated with alteration of climate, is a difficult one, because changes of this type are not easily detected, partly owing to the difficulty of measuring them and partly because of the absence of systematic data. Some idea of their relative importance can, however, be gained.

It would be foolish to deny the possibility that in one or a few cases the Lizard Orchid has occurred in spots which, for some edaphic reason or other, were, in earlier years, closed to it, but it can truthfully be said that there is no evidence of such a thing in the records of the plant. That it frequently occurs on roadsides has already been remarked, and in such spots the likelihood of edaphic change is augmented, but such habitats have occurred over a period of very many years and are in no sense confined to the newer localities. Furthermore, only a proportion of the newer records are associated with such places, and the great majority are from habitats of a kind always and everywhere associated with the plant.

Clearly, if edaphic change is to be invoked as the cause of the sudden spread of the orchid it becomes necessary to postulate a very widespread and simultaneous series of changes all tending towards the same condition, a condition which was, presumably, non-existent or at least very rare previously. It is difficult to imagine anything of this sort, and the records certainly reveal no evidence of it. Sufficiently against it is the observation that while in the last 30 years the plant has been found in many parts of England from which it was hitherto unknown, an even greater number of new records have been noted from within its earlier range and from those regions which have always marked its headquarters.

It may be said, then, that while there is a possibility that individual records of the plant may be due to local edaphic changes this is quite inadequate as an explanation of the general augmentation

of its range and frequency in the last 30 years, or of its great rarity earlier.

The question of climatic change is different, since much attention has been paid to it and elaborate data are available in tabulated form. It is, however, a problem for the skilled meteorologist, and in approaching it I therefore enlisted the help of Dr C. E. P. Brooks, whose work on the climatic changes of the past is so well known. I described to him the three salient features in the history of the Lizard Orchid in England, namely, its virtual restriction to west Kent up to about 1850, its disappearance from here and its extreme general rarity from about 1850 to 1900, and its rapid and wide increase from 1900 to 1933, and enquired whether there were any known climatic changes with which these facts could be correlated. His reply was so interesting that I asked him to recast it in the form of a note that could be quoted here in full. This he did in the following words:

"The Change in the British Climate"

"About 1900 a small but quite definite change occurred in the climate of the British Isles, in the direction of greater 'oceanity'. The change was most noticeable in winter, when the prevailing south-west wind increased in strength and frequency. The average temperature of December to February during the period 1901-30 exceeded that for 1851-1900 by more than 1° F. in eastern, central and southern England and by at least 0.5° F. in all other parts of the British Isles. During the present century there have been no winters comparable in severity with some of the winters of the nineteenth century. The weather of 1901-30 has been stormier and the average rainfall higher; for England and Wales as a whole the three winter months gave an average of 9.52 in. for 1851-1900 and 10.34 in. for 1901-30.

"These characteristics of higher temperatures and heavier rainfall extend into spring (March-May), which was nearly 1° F. warmer in 1901-30 than in 1851-1900 in central and southern England, the rise decreasing northwards to zero in Scotland. The average rainfall of spring in England and Wales was 6.75 in. in 1851-1900 and 7.50 in. in 1901-30.

"In summer, on the other hand, the west winds have been rather more northerly during the present century than formerly, and the average temperature has been about 0.5° F. lower; there has been little change of rainfall. Autumn, on the other hand, has become slightly warmer in England (but not in Scotland) and distinctly

drier, the averages of 1851-1900 and 1901-30 being 10.65 and 10.03 in. respectively. For the year as a whole there has been a slight increase of both temperature and rainfall in England. The general effect of the change is that the climatic zones have moved in an easterly direction across the British Isles."

Since sending me the above note Dr Brooks has published a more detailed account of the facts therein related (Brooks, 1935), and has also drawn my attention to another paper (Bilham, 1933) dealing with some particular aspects of the same subject.

These investigations into climatic change and into the distribution of the Lizard Orchid show that during the last thirty years or so there has been, not only a significant change in certain climatic values in this country, but also a rapid and remarkable increase in the range of the plant. The question now to be considered is how far the relation between the two is that of cause and effect.

It has been shown that the centre of the range of the Lizard Orchid is some degrees farther south than the latitude of southern England and that its occurrence in this country marks the northern limit of its distribution. Furthermore the region in which it occurs most abundantly is in west central France, where the climate is typically oceanic. That this type of climate meets the requirements of the plant more than any other is evidenced by its absence from northern Germany, which is in the same latitude as southern England but where the climate is more continental, especially as regards winter temperature.

Since it is a primary prerequisite for the occurrence of a plant in any area that the climate therein should be suitable for it, it follows that throughout the history of the Lizard Orchid in England there has been some part of this country where it has been able to find the necessary conditions of climate. Moreover, its repeated discovery in new localities during that period indicates sufficiently that there has been no lack of dissemination on the part of the plant. It may therefore be concluded that its very restricted range during most of the period under survey was due to a similar restriction in the distribution of the climatic conditions needed by it.

This being so it further follows that any climatic change which would increase the area over which conditions suitable for the plant prevailed would make possible for it an increased range in this country, and this larger area should, provided that the plant's powers of dissemination and survival remained reasonably constant, gradually become exploited by it. That its dissemination and survival

powers have not appreciably altered is shown by its repeated occurrence in new and widely scattered habitats.

In short, it may be expected that any climatic change calculated to increase the potential range of the plant would be followed by its spread over the area newly made suitable for it, and that such change would be in fact the essential explanation of this spread.

The problem thus becomes the narrower one of deciding whether or not the climatic changes actually recorded are such as to have increased the potential range of the plant in this country.

An examination of Dr Brooks' note shows that the climatic changes therein described are seven in number, namely,

- (1) Increase of winter temperature, with fewer severe frosts.
- (2) Increase of winter rainfall.
- (3) Increase of spring temperature.
- (4) Increase of spring rainfall.
- (5) Smaller decrease of summer temperature.
- (6) Smaller decrease of autumn temperature.
- (7) Decrease of autumn rainfall.

More concisely these may be expressed as two: first, an amelioration of winter and spring temperatures and, second, a slight increase in the preponderance of winter rain. In short the result has been to lessen the climatic differences between eastern England and such parts of the Continent as western France. But in the latter the climatic conditions are, apparently, optimal for the plant, and thus the change of climate in England has been towards the conditions most suitable for the plant, that is to say, the change has been that most likely to result in the enlargement of its English range by increasing the total area wherein suitable conditions occur.

Dr Brooks' closing sentence expresses the total change in rather different words. He says: "The general effect of the change is that the climatic zones have moved in an easterly direction across the British Isles." This is a particularly significant way of putting it, because the climatic differences between the west and east sides of England are essentially, though on a small scale, those between an oceanic and a continental climate. The change thus means that the east of England (where the Lizard Orchid is found) has become less different in type of climate from the west, where the conditions more closely resemble those of western France.

From this it will be seen that there has been since 1900 a concurrent change in climate and in the distribution of the Lizard Orchid, but in addition, what is much more important, that the

former change was such as might provide a reasonable explanation of the latter. There are, in short, grounds for believing that the observed correlation is one of cause and effect.

To one holding, as I do, this view of the matter, the whole problem becomes greatly enhanced in interest in relation to certain theoretical conceptions. A few years ago I gave, in a paper in this journal (Good, 1931), what appears to me to be the explanation of the migrations and mingling of floras so common in the course of geological history. This explanation involved postulating certain relationships between the plant and its surroundings, and these I put forward under the title of the "Theory of Tolerance". I pointed out at the time that, by the nature of the case, circumstantial evidence of the relationships could scarcely be acquired deliberately and might be quite un procurable owing, chiefly, to the length of the time factor involved.

It is in relation to the Theory of Tolerance that the history of the Lizard Orchid in England seems most significant, because it appears to provide an actual instance of the processes comprehended in the theory, and, by an unusual chance, a fragment of that evidence which is normally so hard to obtain.

In terms of the theory I interpret the vicissitudes of the Lizard Orchid in England in the following way.

The climatic values in south-east England before 1900 were just on the border-line of the tolerance of the plant so that it occurred but very locally and occasionally, at times and places when the ordinary minor climatic fluctuations were favourable. When the definite climatic change began in 1900 or thereabouts, this, although comparatively small, was of enormous importance to the plant because it rapidly and considerably increased the area of the country where the conditions were within its tolerance. In short, a comparatively small climatic change had the effect of greatly increasing the potential range of the plant, and this range has subsequently been exploited by it step by step. It is the conception of such processes of cause and effect as this that is the essence of the Theory of Tolerance.

As regards other possible factors, it has several times been suggested to me that at least one in the recent rapid spread of the Lizard Orchid in England has been the increased frequency of its cultivation in gardens. That this may have had some effect on the natural range is possible; but it is important not to over-emphasise its value. First and foremost the cultivation of a plant in gardens cannot in itself

affect the potential range of the plant in a wild state in the neighbourhood. The conditions will there be either suitable or unsuitable according to the climate and soil, and this will not be influenced by the fact that the plant is grown in gardens locally. If the conditions are suitable then the plant may occur wild: if they are unsuitable they will certainly not do so. What garden plants may do, however, is to provide a conveniently local source of dissemination, and they may thereby cause the plant to appear wild before it would otherwise do so. A few of the wild records of the Lizard Orchid may have originated in this way, but even so the fundamental factors underlying the occurrence of the plant in these spots is, not that they have been grown locally in gardens, but that the external conditions are what they require. It certainly must not be assumed that had they not been so grown in gardens their range would never have included that particular neighbourhood. Cultivation therefore must not be regarded as a basic factor in controlling the size of the area eventually occupied by the plant, but it may result in that area, or parts of it, becoming occupied more quickly than might otherwise have been the case.

Even this is probably an over-estimate of the cultivation factor. If the increase in the range and number of wild plants has been accompanied by more frequent cultivation, the latter may well be the effect rather than the cause of the former, owing to the increased notoriety of the orchid. It is also worth noting that the Lizard Orchid is grown in gardens well outside its natural range. Escape or dissemination from gardens would, moreover, be unlikely to result in the gradual westward and northward spread which is so conspicuous a feature of the plant's distribution, or, if it did so, it would be no more than a remarkably interesting substantiation of the fundamental importance of the observed climatic changes. Finally, there is probably little doubt that wild individuals are, in this country, much more numerous than those growing in gardens. If, from these latter, there is subtracted the large proportion which never sets seed, the relative importance of the small remainder shrinks even further.

The actual geographical origin of the disseminules which give rise to new records or occurrences of the orchid cannot be traced accurately, and may, as I have said, sometimes be in garden plants, but it must be remembered that the seeds of the Lizard Orchid are, like others of the family, very minute and likely to be wind-borne for great distances. This, coupled with the persistent predominance of

the plant in numbers near the south-eastern shores of England and its increasing sparsity in all directions inland, inclines me to the personal opinion that a great many of our wild English individuals are the result of direct seed dispersal from the Continent.

SUMMARY

The memoir opens with a detailed account of the distribution of the Lizard Orchid, and this may be summarised as follows:

1. The plant has a wide range over western and central Europe and is particularly common in France, southern Germany and Switzerland.
2. In England it was for many years almost entirely confined to one area only, the immediate vicinity of Dartford in Kent.
3. In the latter half of the nineteenth century the plant was at its rarest in England and was recorded, during that time, from only about 20 localities.
4. Since 1900 it has, in England, spread with marked and increasing rapidity, until by the end of 1933 it had been recorded from a total of 129 localities.
5. In the great majority of localities the plant has been recorded once only, and but rarely has it shown any real persistence.
6. Where the plant has been recorded more than once in the same locality the occurrences have generally been in successive years or with intervals of from 2 to 8 years. Occasionally the intervals have been longer, in one instance exceeding one hundred years.
7. The plant has more often than not occurred as solitary individuals, but more occasionally as from 2 to 12 plants. Only very rarely has it been recorded in anything approaching large numbers.
8. Over 80 per cent. of the English localities are on the chalk. Most of the remainder are on Oolitic deposits.
9. Favourite habitats are on chalk downs, in chalk pits or on chalk railway banks. It has frequently been found by the sides of roads.
10. References to ripe seeds are not infrequent and presumed seedlings have been mentioned.

In the discussion, which occupies the latter part of the memoir, it was shown that, although certain minor features in the occurrence of the Lizard Orchid in England may be attributed to its method of perennation and to the fact that it possesses mycorrhiza, these

cannot explain the main features of its geographical history in this country.

The question was then considered whether the vicissitudes of the plant could be correlated with or attributed to changes in edaphic or climatic conditions, and it was shown to be unlikely that the former had played any appreciable part as a factor.

With regard to climatic change it was shown by the aid of a communication from Dr C. E. P. Brooks that a definite climatic change had occurred about 1900, and that this change had been accompanied by a marked increase in the range of the plant.

The opinion was expressed that this climatic change could be regarded as an adequate explanation of the change in the distribution of the plant in terms of certain views put forward by the author elsewhere under the title of the Theory of Tolerance.

The possible importance of the cultivation of the plant in gardens as a factor in determining its present range was considered, and the conclusion was reached that, while this may occasionally have been the source of individual wild plants, it cannot be regarded as much affecting the larger features of its distribution. The personal opinion was expressed that many occurrences of the plant have been due to direct seed dispersal from the Continent.

CONCLUSION

In the opinion of the writer the conclusions to be drawn from the present and past distribution of the Lizard Orchid and from the subsequent discussion are as follows:

i. The factor which has predominantly influenced and shaped the distribution of the orchid in England is the distribution of climatic values. For many years these values remained more or less constant and were such as to leave but a very small portion of the country open to exploitation by the plant, which during this period was exceedingly local and rare. About the beginning of the present century a change in climatic values took place, with the result that the proportion of the country over which the conditions were suitable for the plant was much increased. This was followed by the rapid spread of the plant into these regions, with the result that in the last 30 years its English range has been greatly enlarged. This change of climate may be regarded as the cause of the change in distribution, and the history of the plant in England thus appears to afford a small but definite piece of evidence in support of the Theory of Tolerance.

2. Factors of soil condition have had no more than a local influence on the distribution of the plant.

3. A small number of wild English individuals may have originated from plants grown locally in gardens, but most of the records appear to be of natural origin, and many suggest that they are the result of direct seed dispersal from the Continent.

4. The facts that the plant possesses mycorrhiza and perennates by tubers are regarded as explaining certain common minor features in its occurrences, and especially its usual failure to persist in any one place for more than a few seasons.

ACKNOWLEDGMENTS

The compilation of this memoir has been possible only by the kind co-operation of a very large number of persons, and it is a great pleasure to acknowledge gratefully here all the help that I have received from them. To all helpers my thanks are due, but I would express my special indebtedness to Dr C. E. P. Brooks, Mr P. M. Hall, Mr W. H. Pearsall, Mr C. B. Tahourdin, Dr M. A. H. Tincker and Prof. F. E. Weiss.

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REVIEWS

Botany as an Experimental Science in Laboratory and Garden. By LILIAN J. CLARKE, D.Sc., F.L.S. Pp. xii+135, with 9 plates and 21 figures in the text. Oxford: University Press. 1935. 6s. net.

This is not a text-book but a record of the work done (since 1896) in the laboratory and garden of the James Allen Girls' School, Dulwich, under the direction of (the late) Dr Lilian J. Clarke.

In a foreword Prof. Tansley pays a well-deserved tribute to Miss Clarke for "her thoroughly sound fundamental ideas, her extremely clear and honest mind, her keen enthusiasm, and her indomitable energy and perseverance". All teachers will endorse this, and few (if any) will regret that she did not write a regular, practical handbook for school laboratory and garden work rather than a record of her work at Dulwich. The material is there, as Prof. Tansley says, and can be extracted and used to great advantage in text-books; but this is more than a "mere textbook"—it is a notable contribution to the practice of education.

Dr Clarke's attitude towards the teaching of biological science is defined in the preface where she quotes Huxley, and her work at Dulwich must be interpreted in the light of this statement: "The subject matter [of biological science] is different from that of other sciences, but the methods of all are identical; and these methods are:

- "1. Observation of facts, including experiments.
- "2. Comparison and classification, the results of the process being named General Propositions.
- "3. Deduction.
- "4. Verification.

"Such are the methods of all science whatsoever."

Her work in the laboratory and the school does not differ materially from that done in some other schools during the same period: it was an attempt to put into practice some well-established precepts, as, for example, "learning by discovery", and its corollary, "no text-books in the early stage"; but she differed from the rest of us in the assiduity with which she pursued her ideals.

Teaching is always a compromise between the ideal and the practicable. To the ideal teacher working under favourable conditions the school text-book may not be a necessity, but to most of us under existing conditions it is quite indispensable. There is nothing educationally unsound in its use: it is its misuse that causes all the damage. Slavish adherence to the text—to the selected matter, to its form and to the method and order of its presentation—must lead to harmful results. In the hands of a good teacher, the text-book is used much in the same way as the "records" are used at Dulwich, i.e. for purposes of reference and comparison with the results obtained by the pupils themselves.

In order to pursue her ideals a step farther, Miss Clarke gradually converted the school grounds into a botany garden, and eventually built in it a collection of ecological habitats—an effort, as far as the writer knows, that was unique in this country.

The story of the evolution of this botany garden from small beginnings with single plants, "order" beds, plots for soil, pollination, and Mendelian experiments, culminating in "ecological habitats"—the lane and wall, the pond, the heath and bog, sand-dune saltmarsh, and pebble beach, the cornfield

meadow and chalk bed, and the wood, is an epitome of the development of botanical thought during the last thirty years.

With the development of the ecological outlook, the teacher's difficulties with respect to the organisation of school work have increased enormously. In the past, when the work was concerned mainly with plants as individuals, occasional rambles into the country sufficed, for the necessary material could be brought to the school, or grown in the school garden; but with the growth of the study of plants in communities, this transference of material to the school is (normally) impossible, and (so to speak) the "school" has to be taken to the plant community.

For schools in rural areas, the problem is relatively simple: the whole of the "local" area, i.e. the country within easy reach of the school, is regarded as a "school garden" or "experimental plot", and practically the whole work of the school in botany may be based on the study of one or more communities within easy reach.

In urban areas, especially in schools in industrial districts, where there are no plant communities of any kind, the teacher, speaking generally, is forced to fall back upon the old plan—occasional visits to the country. There is, however, one method by which this occasional work on natural communities may be supplemented, viz. the use of artificial communities, built in a setting as like the natural as possible, in the school garden (if any) or on any available plot of land in the vicinity of the school.

This was Lilian Clarke's way, and with characteristic energy and at some expense, she "imported" material from natural communities in the country, and thus the remarkable collection of "ecological habitats" at Dulwich came into being.

In short, she preferred to train the powers of observation, deduction, etc., of her pupils by means of (relatively) inferior material of "made" communities close at hand and under control rather than risk the possibility of casual observation and unsound deduction, hurriedly made, from good material in natural communities far away. And rightly so, provided that there is as little interference as possible with the "made" communities (no undue tidying up!), and above all that the pupils make periodical visits to similar communities in their natural homes, for (we believe) that in this way only can the ecological outlook be fully developed.

Botany as an Experimental Science in Laboratory and Garden should be in the hands of all the teachers of biology, not primarily for the matter therein or even for its method, but because it is a record of the life-work of a great teacher—a record the perusal of which should engender the spirit of true adventure so necessary in the practice of teaching.

E. PRICE EVANS.

Limnology. By PAUL S. WELCH. Pp. 470, with 46 text-figures. New York and London: McGraw-Hill Book Company. 30s.

Originally concerned mainly with the hydrology of lakes, the content of limnology has steadily expanded until now it deals with the whole subject of inland lakes and their biology and is even extended to include ponds, streams and rivers. It is this extended use of the term which is employed in this book. With the extension of the subject matter has come an ever-widening interest in and an ever-increasing literature on the subject, especially in its biological aspects; so that undoubtedly the time is ripe for an up-to-date treatment in English. Many will, therefore, turn to this book with a good deal of interest, the more so, perhaps, because its author has been associated with the development of the subject in the United States where there has been considerable limnological activity in the last twenty years.

Many, no doubt, of those interested in limnology will open this book in the hope of finding some general survey of North American results and, particularly, some attempt at co-ordinating these results with European work. In these respects, their examination is likely to meet with mixed success, since, on the whole, the book is designed primarily as a text-book and a work of reference. Little in the way of wider integration of existing results is attempted, apart from that already familiar in standard works on the subject. As a survey of existing evidence, however, the book will undoubtedly prove useful, particularly, perhaps, as a source of information on North American waters. It is well supplied with references and it touches on most of the wide range of subjects which falls within the province of the limnologist.

It is possible that, while recognising these virtues, the reader may share with the reviewer a feeling of some disappointment. Apart from the difficulty of formulating any clear picture of North American lake types and their biota, there is distinctly uneven treatment of many sections of the survey. The botanist will undoubtedly tend to be especially critical in this respect, for although adequate attention is given to the fungi and bacteria, the far more fully investigated plankton algae receive only passing attention and the benthic or littoral algae are dismissed in a short paragraph. Such weaknesses might be tolerated if the book professed to be written mainly from the zoological aspect of the subject. The author, however, defines limnology "as that branch of science which deals with the biological productivity of inland waters and with all of the causal influences which determine it". It is thus difficult to overlook the somewhat scanty attention given to the algae and, in particular, it is remarkable that there appears to be no reference to modern views as to the importance of the nanoplankton as agents in biological productivity. Certain parts of the chemistry underlying production in lakes are similarly treated in a very bare manner, notably the chemical changes ensuing during stagnation and circulation. These are discussed almost entirely as changes in the dissolved gases. Such features as these leave one with a feeling that emphasis on biological productivity as the basis of limnology is likely to lead the investigator or student to look at the end of the biological sequence without adequate analysis of the causal steps involved in attaining that end.

W. H. PEARSALL.

Plant Physiology. By MEIRION THOMAS. 8 x 5 in. Pp. xii + 494, with 57 text-figures. London: J. and A. Churchill. 1935. 15s.

This is the first text-book of plant physiology for advanced students to be published in this country for a good many years. As such it will inevitably rouse many hopes and some fears among all who are interested in the subject.

The author's aim has been to fill in the gap between a first introduction to the subject and the monographic reading of the specialist; a certain elementary knowledge is assumed, and at the other extreme no attempt is made to say the last word on any topic. The appetite is aroused, but not surfeited with a glut of argument, instance and deduction. The subject is treated in four parts; two appendices supply notes on the chemistry of metabolic products and on physical chemistry respectively, while a third contains a selected bibliography and a satisfactory index. The separation of the chemistry and physics from the general text is a method which some will approve and others probably dislike. A good deal of cross reference inevitably results, but a more concise text is obtained.

The four parts of the book proper deal with (1) protoplasm, (2) the absorption, translocation and elimination of water, solutes, and gases, (3) nutrition and metabolism, (4) growth and movement.

The treatment is uniform in that a sound rather than an "up to the minute" treatment is aimed at throughout, great restraint is always exercised in dealing with current theories (whether attractive or repulsive), and yet the pitfall of a colourless and non-committal catalogue of facts is avoided. The last can be attributed to the author's concern always to trace physiological phenomena to their physico-chemical bases. He admits that this has forced upon him a slender treatment of some of the more purely descriptive branches of the subject, such as development and reproduction, but the gain in unity and logical connection of ideas is very considerable.

One of the most striking features of this book is its ventilation of basal conceptions that are generally slurred over or even completely ignored in physiological summaries. Noteworthy examples are the sections describing the general methods for testing metabolic hypotheses, and on the ordered metabolism of whole cells as contrasted with enzyme mixtures. In his choice of material the author seems to have been remarkably successful. There is very little that anybody is likely to wish away, and, having due regard to the difficulties of choosing, not much omitted that could reasonably be looked for; standard views are faithfully represented. We must confess to a little disappointment at the almost total neglect of the soil nutrients, but in view of the admitted difficulty of the subject, this will be regarded as a hard opportunity lost rather than a duty neglected.

Controversial statements are infrequent, but on p. 199 the use of the words "diffusion gradient" and "diffusible" in connection with the formation of metabolites seems to suggest more than was perhaps intended. The carbon dioxide of respiration is not likely to be used up wholly by photosynthesis (p. 142) since illuminated leaves in a carbon-dioxide-free atmosphere can be shown to be giving off the gas. It may further be remarked that roots are not likely to grow in pots in the manner shown by Figs. 6 and 8. A fault of presentation that reaches serious proportions is the excessive use of footnotes, going to the length on p. 285 of a footnote to a footnote. It is sincerely to be hoped that an opportunity will arise of incorporating the great majority of these into the text where they clearly belong. The very great and obvious utility of the book to young students of plant physiology should both justify and make possible such minor improvements to what may well become a standard text for some years.

W. O. JAMES.

Intermediate Botany. By L. J. F. BRIMBLE. Pp. 562. London: Macmillan and Co. Ltd. 1936. 8s. 6d.

This book is intended for students taking Higher School Certificate and other examinations of the same standard. Two-thirds of its thirty chapters deal with the anatomy and physiology of flowering plants; of the remainder, three are historical and introductory, four are on the lower plants, one is on ecology, and one on evolution and on variation.

There are many other books which cover the same ground, and we therefore expect this to justify its publication by the possession of special merits.

Such merits are not entirely absent; the diagrams are on the whole very good, and the distinction between a diagram and a drawing is made quite clear. Also, some passages, such as that on suction pressure, are well written. We have to set against this the fact that badly written sentences occur on practically every page; and that the author says many silly things which he does not mean, such as: "The hydrogen-ion concentration varies with the enzyme."

But the book's most serious fault is the treatment of plant structure from the teleological viewpoint, for example, "The root performs several functions, the two chief of which are to anchor the plant...and to absorb water." If the reader accepts this statement, he accepts as well a philosophy incongruous with scientific thought in general. If, on the other hand, one says that the plant

absorbs water through its roots, one is stating a fact which can be demonstrated by experiment, and no more; and an elementary text-book should aim at presenting such facts in a straightforward way. It is only too common, at the present time, to meet students who have come up to the university with a knowledge of physiological anatomy which has no sound basis in experimental physiology; and recent work on xerophytes has shown how inaccurate this kind of knowledge may be.

We must point out, in addition, a few of the many errors and omissions. The word equilibrium is missing from the chapter on enzymes; it is said that stipe and frond in *Fucus* are structurally the same; though both auxin and phototropism are mentioned, it is clear that the author does not realise that there is a relation between them; and the ages of the three great geological eras are given as three, nine and eighteen million years respectively. We throw in the thoroughly confused account of Mendelism as make-weight to this collection.

The author, in his preface, says that he has attempted to make the book a help to "the general, scientifically interested reader". The inadequate treatment of evolution, to give only one example, shows how little the attempt can be a success; and we venture to prophesy that the book will be used, if at all, as a cram-book, and that it will be deemed, by the general and the critical reader alike, to be unscientific and redundant.

D. H. VALENTINE.

Le Genre Galera (Fr.) Quellet. Encyclopédie Mycologique. By R. KÜHNER. Vol. VII. Pp. 240, 8vo. (16×27 cm.), with 75 text-figs. Paris: Paul Lechevalier. October 30, 1935. 75 fr.

Under this misleading title the author describes some fifty species of agarics which he has collected in France and Algiers and which he assigns to the genera *Conocybe* and *Galerina*. The book is not the monograph of *Galera* we had hoped for; only a few other species from North America and Central Europe are mentioned. After a critical account of the previous attempts at classifying these small toadstools, the author elaborates a new system which follows the lines of Fayod's Prodrome and is based principally on the microscopic structure of the surface of the pileus and on the cystidia. The limits and affinity of the genera *Conocybe* and *Galerina* are discussed in separate chapters, but with little conviction because our knowledge of the structure of the Ochrosporae is too meagre. The greater part of the book is filled with lengthy descriptions, based mostly on the author's collections. Seven species and sixteen varieties are described as new. There is a wealth of detail about the spores, cystidia and gill attachment, all of which features are illustrated for each species. The spores are drawn higgledy-piggledy and cannot be compared accurately without tracing them off. The basidia of most species are drawn with three sterigmata; in the descriptions they are said to have four.

By revealing how many diagnostic features have been overlooked by mycologists in studying this difficult group, how variable the species may be and how important it is to describe the microscopic structure of the pileus, the author has done this branch of systematic mycology considerable service. But we wonder why the author has been content with "un simple scalp du chapeau". Little by little does the systematic study of agarics progress. The complete pocket-lens system of Fries, still the most satisfactory and practical, is being dragged into chaos by the fragmentary study of squashed gills and "petites coupes transversales et tangentiales" of the pileus. Any striking configuration of the hyphae becomes the basis of a new genus without the development of the tissue and the manner of its variation in the family being understood. For these reasons and because the development of the fruit-body is wholly ignored and the microscopic structure of the majority of the species in the world unknown,

we consider the author's new classification untimely, unsubstantiated and muddled. The author has emended *Conocybe* Fayod to include *Galera tenera* and *Pholiota togularis* and to exclude *Galera hypnorum* and *Pholiota marginata*; *Galerina* Earle he has emended to include *Galerina hypnorum*, *Naucoria triscopoda*, *Tubaria paludosa* and *Pholiota marginata* and to exclude *Tubaria furfuracea*. But why species with and without a veil, species with a straight margin to the pileus and an incurved margin, species with annexed and decurrent gills, and species with cartilaginous and fibrous stems should be placed in the same genus, contrary to accepted principles, the author does not explain. No reasons are given why *Pholiota togularis* is taken to be more nearly related to *Galera tenera* than to *Pholiota praecox*, and *Pholiota marginata* to *Galera hypnorum* rather than to *Pholiota mutabilis*. The supposed absence of cystidia from *Cortinarius* subgen. *Telamonia* and *Hydrocybe* is the sole means of distinguishing these subgenera from the comprehensive *Galerina*. We are far from convinced that the difference in surface structure of the pileus between *Conocybe* and *Galerina* has the significance which the author assumes because the same difference, together with every intermediate state, can be found among obviously allied species of such diverse genera as *Pluteus*, *Russula*, *Hygrophorus* and *Boletus*. If we understand rightly, the difference depends on whether the hyphal ends on the pileus inflate into clavate pileocystidia or not. When exactly the same difference is found among the hyphal ends on the stem the author sees in it a character to distinguish only subsections of a section of a subgenus of the genus *Conocybe*, cf. the *Capitatae*. Inoderm pilei, moreover, may have pileocystidia in the primordium which collapse on the expansion of the fruit-body. The author's figure of the apparently inoderm *Galerina clavata* (Fig. 2) suggests that its primordial pileus has a palisade which, if not so well developed as in typical *Conocybe*, is somewhat intermediate between the two extreme states. Lack of consideration is shown also in the author's habit of often giving two lengthy descriptions for one species and leaving the reader to discover for himself what difference there may be between them. We find, too, *Galera hypnorum* Fr. sensu Rea (Brit. Basid.) cited, without proof, as a synonym of *G. rubiginosa* Fr., as though British mycologists with the Friesian tradition handed down through field excursions from Berkeley's day did not know that common species. As the author cannot recognise *G. hypnorum bryorum* we suspect that the mistake is his. We would question several other of the author's interpretations of Friesian species which have become well established by tradition in the field.

Our chief criticism lies, however, in the author's disregard of the International Rules of Botanical Nomenclature, 1935, the effect of which is both to invalidate his new species, varieties, sections, subgenera and emended genera and to create many unnecessary and impossible new combinations. As there is no Latin diagnosis in the book, so the new descriptions are worthless. The genus *Galera* (Fr.) Quélet, which the author deposes as a later homonym (cf. *Galera* Bl., Orchidaceae) in favour of *Conocybe* Fayod, is given in the Rules as a *nomen conservandum* and *Conocybe* as a *nomen rejiciendum*. This disregard is, perhaps, the reason why *Conocybe* is divided into subgenera and *Galerina* into sections, and why some species are described with varieties and forms and others with forms without varieties. The index is confusion worse confounded. All the species of *Conocybe* and *Galerina* are listed under *Galera*, thus making, for the first time, new combinations in nomenclature for both the old and the supposedly new species, e.g. *Pholiota marginata* (Batsch) Fr. described as *Galerina marginata* (Fr. ex Batsch) Kühner and indexed as *Galera marginata* Batsch (!), and *Conocybe subnuda* n.sp. indexed as *Galera subnuda* Kühner. There are also such misleading citations as *Galera vittaeformis* Ricken, *Naucoria sideroides* Ricken and *Galera hypnorum* Rea.

Proper regard for scientific procedure by the author would have made this book worthy of its publisher and most useful throughout to the systematic mycologist.

E. J. H. CORNER.

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STUDIES IN THE AUTECOLOGY OF *CLADIUM MARISCUS* R.BR.

I. STRUCTURE AND DEVELOPMENT

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(With Plate II and 16 figures in the text)

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I. INTRODUCTION

IN some floras *Cladium Mariscus* is stated to be rare in England, but in places it occurs in considerable abundance while it is found locally in most counties. Its greatest abundance is in the Norfolk Broads district, and it is often to be met with in the marshes and fens of East Anglia. In particular it covers large areas of Wicken Fen, Cambs., and the plant communities to which it belongs have been described and discussed by Yapp (1908), Godwin and Tansley (1929), Godwin (1931), Godwin and Bharucha (1932). Yapp (1908)

has given some account of the growth form of the plant in the Fen. It is here that its autecology has been studied in more detail.

The water-table in the fen is close to the surface of the soil for a large part of most years, so that the roots of many of the plants are growing in waterlogged conditions. It is a matter of some interest to know what relation this habitat factor bears to the species which occupy the habitat, and *Cladium Mariscus* was chosen as a suitable species to investigate from this point of view. A number of laboratory experiments were carried out to find out the nature of the inter-cellular air-space system of the plant, and the effect on the plant of varying the oxygen concentration surrounding the roots. It was also noticed that the species showed interesting points in growth and development, and this led to a study of growth-rates of the plant under different conditions and an attempt to make some analysis of growth phenomena that might be of general interest. Other aspects of the autecology of the species have also been considered, but the results of two years' work on the species may be said to relate substantially to these two main issues, namely the mechanism of aeration of the subterranean parts of the plant, and the factors which influence its growth-rate.

Neither of these aspects can be considered independently of the physical construction of the plant, and hence before either set of results can be interpreted some account of it is desirable. The present paper is therefore a necessary preliminary to the publication of the more specialised lines of work which diverge from this central starting-point. It must be emphasised that this paper has not been written from the point of view of an anatomist or morphologist, and hence it does not attempt a full account of the anatomy of the species. For the most part only those points have been dealt with which are of evident importance for the physiological and biological problems in view, particularly those of "aeration" and growth.

II. OUTLINE OF DEVELOPMENT

The seedling stages of *Cladium Mariscus* are unknown to the writer, so that this description starts with the appearance of a bud on the underground stock of the parent plant (Text-fig. 1 B). This bud grows horizontally through the soil for a distance which varies from 1 to 20 cm. The apex then bends upwards and the embryonic leaves in the bud elongate. The outermost are short and brown, but the inner ones are longer and become green when they reach the soil

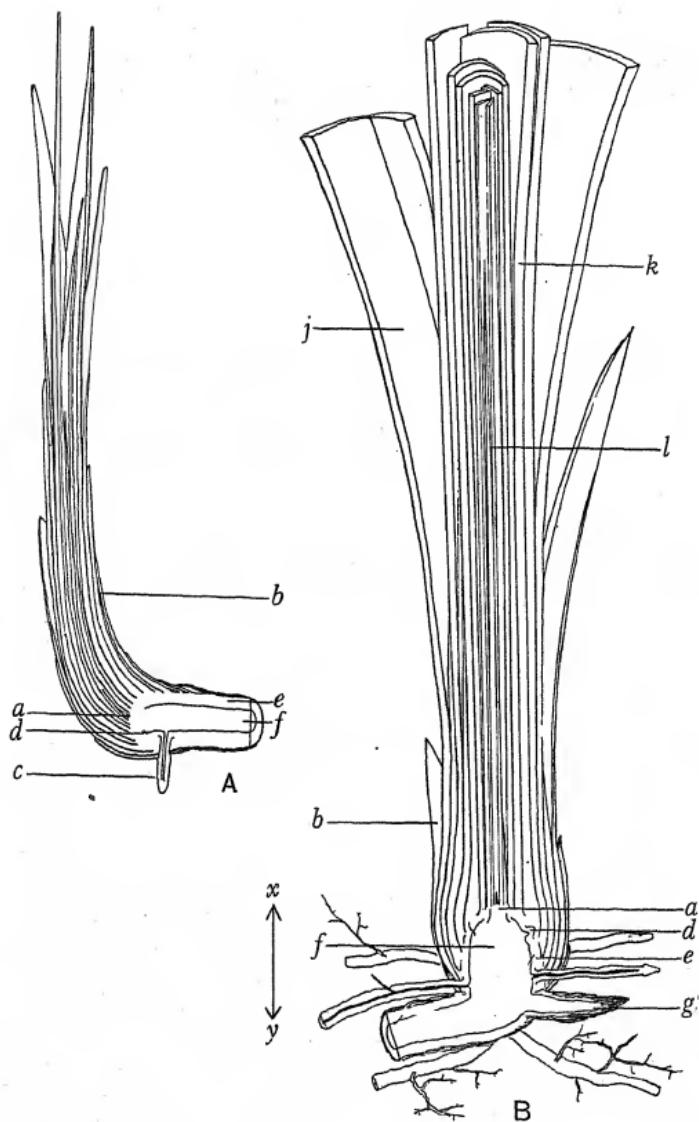
surface. Meanwhile roots are put out from the right-angle bend of the rhizome just below the growing-point. It is from this region also that new buds grow out, thus repeating the process of vegetative reproduction. It is comparatively rare to find buds produced later than the first or second year of the growth of the shoot. The result of this is that the young rhizome lies at about the same depth in the soil as that which gave rise to the parent plant. This depth varies according to the situation, but in the Cladio-Molinietum at Wicken Fen it is between 10 and 20 cm. The rhizomatous habit of the plant has been clearly shown by Yapp (1908) in his Text-fig. 12, p. 73.

Once the bud has turned upwards new leaves are continually produced, which grow to a height which is roughly the same for all the leaves of one plant, but which varies with the habitat. In the luxuriant growth seen in the Norfolk Broads the leaves may be as much as 3 m. high; in poorly grown specimens they may be as little as 70 cm. They arise centripetally with a phyllotaxy of $1/3$, and as new ones appear the older ones to the outside gradually die. The shoot apex grows upwards slowly as the number of leaves increases so that an upright root-stock is formed. This part of the plant will be called the *stock*, whereas the horizontal stem structure which connects the young shoot to the parent will be called the *rhizome*. Numerous roots are produced from the stock, always arising close behind the apex. To reach the soil they have to penetrate the bases of the older leaves, and where this is difficult a root may grow upwards between the leaves for a distance of 10 cm. or more. Text-fig. 1 A shows a young shoot cut in half longitudinally. The leaves have just grown out to the level of the soil surface; they would normally be packed close together into a spike, but have been shown separated in the diagram for the sake of clearness.

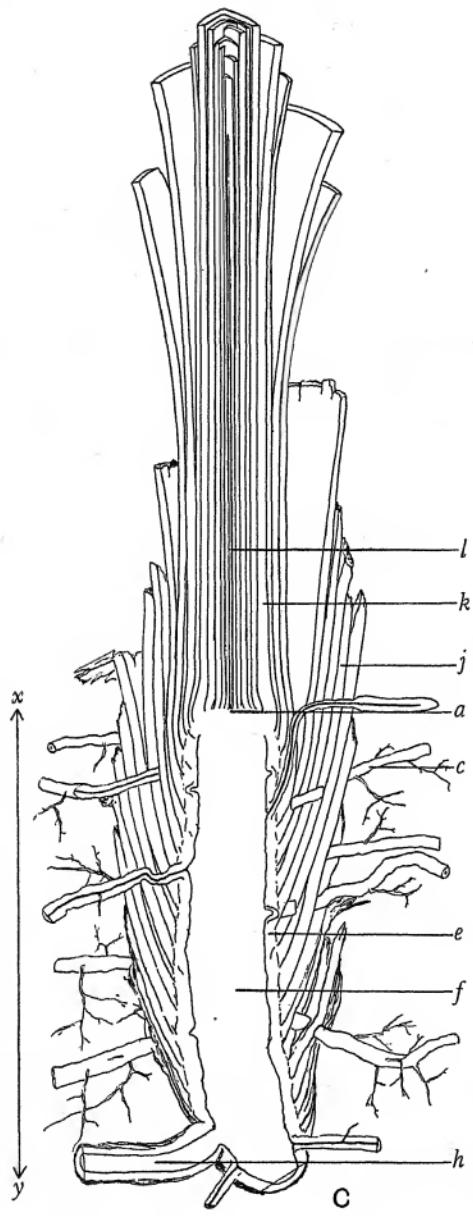
Text-fig. 1 B shows a slightly later stage of development than Text-fig. 1 A: a short length (*xy*) of stock has been formed and from it a number of roots have grown out. At *g* is shown a bud just starting to grow out. This will grow into a shoot of the next generation.

In Text-fig. 1 C the stock is considerably longer (*xy*) and many of the older leaves have rotted away, leaving only their dead bases.

When flowering occurs, the growing-point does not remain below ground, but shoots up as the flowering stem, which bears leaves similar to those produced on the stock, though shorter. The stem is terminated by a much-branched panicle. After flowering the whole shoot dies; by the time the seeds are shed, all the leaves on the stock have turned brown and the flower stem withers soon after.



Text-fig. 1. Shoots of *Cladium Mariscus* cut in half longitudinally. A, young shoot; the leaves have been slightly separated for clearness. B, shoot a year old; a bud has been produced. C, shoot 4 or 5 years old. a, shoot apex; b, brown scale leaves; c, root; d, root initial; e, cortex, f, stele of stock or rhizome; g, bud; h, rhizome of new shoot; j, leaves completely brown; k, leaves green but not growing; l, growing leaves; xy, represents the length of the stock.



In the conditions under which the plant grows at Wicken Fen, it often happens that a shoot does not flower at all. The shoot continues to grow vegetatively for a certain length of time, and then becomes moribund. Data have been collected which suggest that 8-10 years is the average length of life in the Cladio-Molinietum. On digging up a living shoot the soil around it is usually found to contain the rotting bases of two or three dead shoots. No reason is at present known for this apparent senescence, nor is it known what conditions determine whether or not a shoot shall flower. In general it may be said that a *Cladium* community flowers much more freely when it is growing in open water than in the more raised ground which forms the largest habitat for the plant at Wicken Fen. Other factors besides the level of the water-table probably have some effect since the degree of flowering varies very greatly according to the season; for instance in 1934 the species flowered freely in the Fen, whereas in 1935 not a single flower was found.

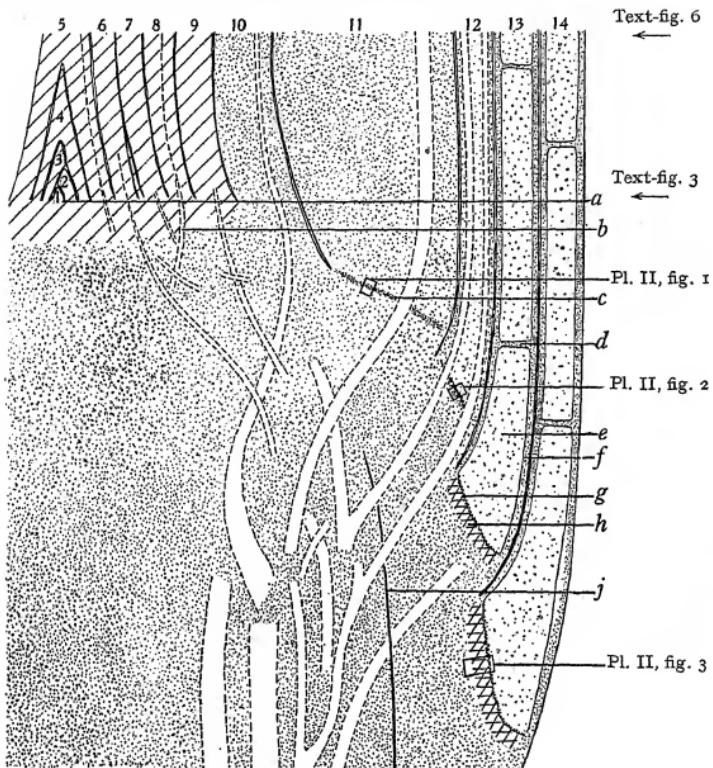
III. THE GROWING-POINT AND THE PRODUCTION OF LEAVES

Text-fig. 2 shows one side of the stock of a shoot. It is a schematised drawing of an actual thick longitudinal section which includes the growing-point (*a*). It is impossible to construct an idealised diagram of this region because the course of the vascular bundles of the stock varies a great deal. Nor could it be shown completely in a vertical section because the bundles branch and do not keep to one vertical plane for long distances.

The figure therefore shows the parts of vascular bundles that were actually seen in the section. The central cone at the growing point is taken as leaf 1, and the remainder are numbered in order of increasing age. In this species, as in many Monocotyledons, growth of the leaf is basipetal so that there is a meristematic region in the lowest part of the lamina. In Text-fig. 2 the meristematic regions are hatched; in the outer leaves which show no meristem, growth has ceased. Leaf 10 may still be growing at a slow rate compared to the inner leaves, but the meristematic activity has almost ceased.

At the stem apex are found the leaf rudiments. They are packed close together to form a conical central mass 1 or 2 mm. in height, and they consist entirely of meristematic cells. It is usual to find that the leaf just outside the rudimentary leaves (i.e. leaf 5 in Text-fig. 2) has already grown out to a length which may be as much as 20 cm., and hence there is a fairly sharp distinction between "rudimentary" leaves and those which are actively elongating.

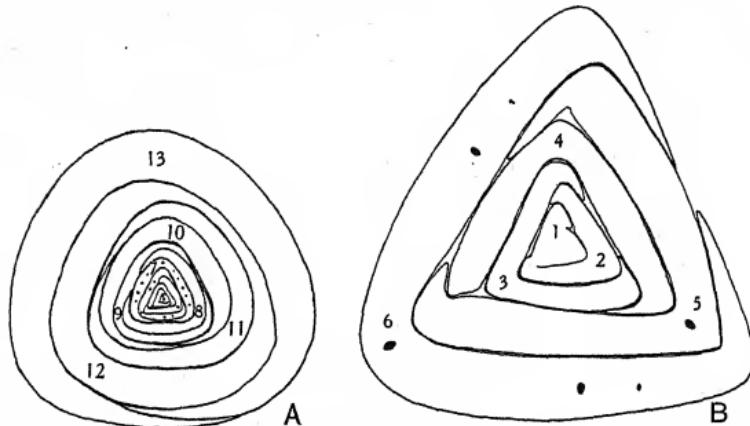
Growth of the leaf takes place almost entirely in a vertical direction, so that the leaf rudiment and the mature leaf have more or less



Text-fig. 2. Schematised drawing of an actual thick longitudinal section of one side of the stock apex. Meristematic regions hatched. Outlines of vascular bundles indicated by dotted lines. Leaves numbered in order of increasing age: 1-4, type A; 5-9, type B; 10-12, type C; 13 and 14, type D (see p. 189). *a*, growing-point; *b*, protoxylem strand; *c*, zone of more closely packed cells; *d*, horizontal septum; *e*, pith of leaf; *f*, leaf palisade; *g*, collapsed pith cells; *h*, lignified cortical cells; *j*, starch sheath. The levels of the sections shown in Text-figs. 3 and 6 are indicated, and also the regions shown in detail in Pl. II.

the same shape in section. Owing, however, to the cell divisions occurring in the stock itself at the bases of the younger leaves, a certain amount of tissue is added to the base of the leaf during the

time it is elongating. Text-fig. 3 A shows a transverse section taken across the growing-point of another plant at the level indicated in Text-fig. 2, and Text-fig. 3 B shows the innermost region of Text-fig. 3 A on a larger scale. This plant had a larger number of actively growing leaves than the plant from which Text-fig. 2 was drawn, so that while in both figures leaves 1-9 may be taken to correspond with each other, leaves 10 and 11 of Text-fig. 2 correspond to leaves 12 and 13 of Text-fig. 3. Leaf 4 is the largest of the rudimentary leaves and its base does not entirely enclose the growing-point. Leaf 6 has the lateral wings much extended, and in leaf 11 of Text-fig. 3 they enclose

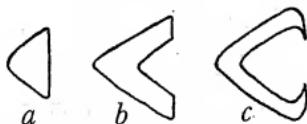


Text-fig. 3. A, transverse section across a shoot at the level indicated in Text-fig. 2. B, enlarged drawing of central region of A. Leaves numbered from within outwards. Position of protoxylem strands indicated by black dots for all leaves up to no. 7.

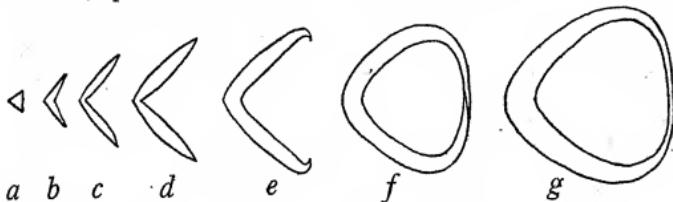
the growing-point and overlap one another. The direction of this overlap is the same for all the leaves of one shoot but varies from shoot to shoot. Leaf 12 in Text-fig. 3 shows a continuous ring of tissue: in a section a few cells higher there is an oblique line of separation.

A leaf rudiment cut transversely at different levels would show a series of sections such as those in Text-fig. 4. Section *a* is the most distal, *c* the most proximal. When extension of the cells sets in it takes place at the upper end of the meristematic region, and hence the first part of the leaf to differentiate will be the apical part with a triangular section; the part with a V-shaped region will elongate next and finally the region with the V-shape and inturned margins.

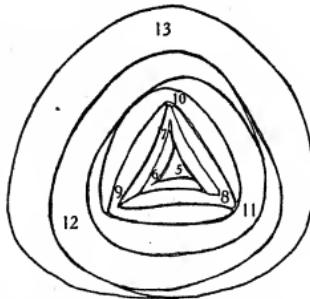
Hence a leaf which has completed its growth, say 1-2 m. long, shows a series of cross-sections along its length, illustrated by Text-fig. 5. The last two sections (*f* and *g*) are not represented in the rudimentary leaf because during leaf development the basal meristem is further



Text-fig. 4. Shape in section of a rudimentary leaf at different levels. *a* is distal, *c* proximal.



Text-fig. 5. Shape in section of a mature leaf at different levels. Distances in centimetres from the base are: *a*, 200; *b*, 170; *c*, 130; *d*, 90; *e*, 30; *f*, 5; *g*, 0.



Text-fig. 6. Transverse section across the same shoot as that shown in Text-fig. 3, taken 1 mm. higher up, at the level indicated in Text-fig. 2. The numbers of the leaves correspond to those in Text-fig. 3.

modified in form, itself taking on an annular section. This is evidently due to enlargement by addition from the meristem of the stock.

Text-fig. 6 is a section across the same shoot as that shown in Text-fig. 3 but taken about 1 mm. higher; the level is indicated in Text-fig. 2. It differs from the section of Text-fig. 3 in that the centre is filled by the triangular part of leaf 5. Leaves 6-10 also show a

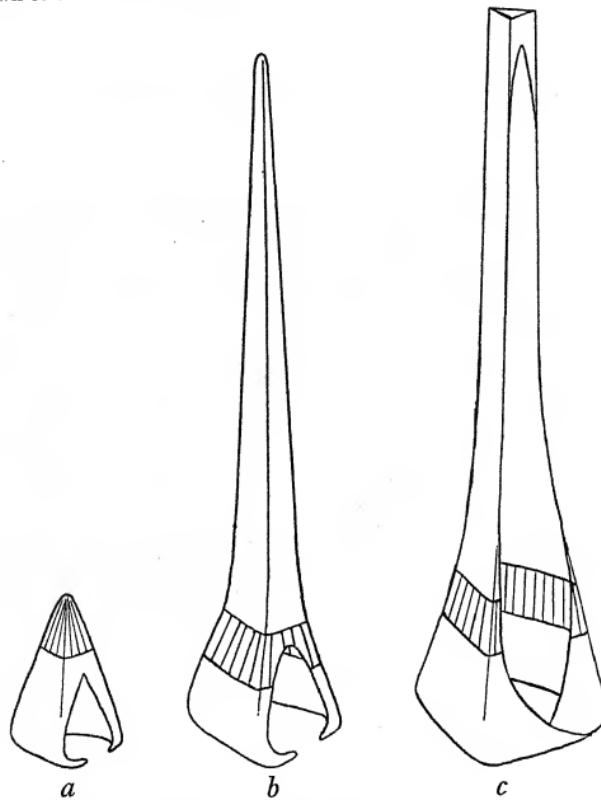
strictly V-shaped section in contrast to the shapes shown in Text fig. 3.

It seems as though the main growth activity of the leaf took place in the upper part of the meristematic region at a distance of at least 1 mm. from the stock. Several facts suggest this: firstly there seems to be a considerable interval between the time at which leaf rudiments are first formed and the time at which they start to elongate rapidly. Secondly, a leaf such as no. 6 in Text-figs. 2, 3 and 6, may have a total height of 30 cm., and the inturned wings are already formed at its base, yet probably another 50 cm. of lamina having a V-shaped section will grow and differentiate before the most basal part increases in length. Again, although there may be a region at the very base of the lamina where the leaf tissues form a continuous ring, this region is never more than a few cells in height. In other words, this region never grows upwards appreciably even when the leaf is fully mature. The author visualises each leaf rudiment as potentially meristematic throughout: strong meristematic activity does not, however, affect the whole tissue at the same time, but a wave of maximum activity starts at the apex and progresses slowly towards the base, producing a considerable length of mature leaf representative of the cross-section of the rudiment at successive depths. Before actually reaching the base where the leaf tissue is encircling, the wave of activity dies out and differentiation of the meristem sets in—in other words, meristematic activity ceases. Thus no part of the leaf ever has a completely annular cross-section.

These facts may be summarised by a diagram (Text-fig. 7 *a, b, c*) illustrating the history of an individual leaf. The horizontal scale is much larger than the vertical. In each case the shaded region is the one in which rapid growth is about to take place. *a* is still rudimentary, but in *b* considerable vertical growth has occurred; *c* is taller still, and only the lower half is shown. *c* shows how the shape of the basal attachment has changed owing to the addition of tissue to the leaf by the meristematic cells of the stock. Text-fig. 7 also shows that, as each leaf grows older its attachment to the stock becomes pushed farther and farther away from the growing-point, while new leaves are continually being thrust up within it.

Differentiation of the leaves takes place from above downwards, and is usually completed as far as external shape and texture are concerned, a few millimetres above the base. Growth keeps pace with differentiation for some time, but when the base of a leaf has been pushed out so that it reaches a position such as that of no. 10

in Text-fig. 2, either the differentiation rate increases or the growth-rate decreases, the net result being that differentiation proceeds right down to the base of the leaf.



Text-fig. 7. Drawings to illustrate the growth and development of an individual leaf. In each case the region which is about to grow rapidly is shaded. The horizontal scale is larger than the vertical.

The rate of upward growth of the actively growing leaves (5-9 in Text-fig. 2) shows a most striking feature in that at any one time it is the same for all of them. The actual magnitude of this common growth-rate varies considerably, yet all these leaves are so closely similar in their extension that if the upper parts of the leaves are all

cut off at the same level, many weeks later the cut ends will be at the same height to within 1 mm., though they may all have grown up 70–100 cm.

The common growth-rate of these inner leaves shows a marked seasonal periodicity. In the field conditions at Wicken Fen these leaves do not grow more than 1 or 2 cm. between October and March, whereas they may grow as much as 100 cm. in the summer months. Any particular leaf has an active meristem at its base for about two growing seasons. For instance, if leaf 4 in Text-fig. 2 starts to elongate rapidly in July of 1935 it will continue to do so till October 1935, then pause till the growing season of 1936, grow throughout that, and finally lose its meristem in June or July of the following summer. Similarly, one that started in March 1935 would cease to grow in October 1936. The change in behaviour thus appears to be correlated with the position of the leaf relative to the stem apex, for it may occur at any time during the growing season. The time taken for the growth-rate to fall to zero varies from 3 to 6 weeks, so that it is fairly short compared to the time of active growth. Leaves at this stage often show swollen bases as is illustrated by leaf 11 of Text-fig. 2, and by some of the leaves in Text-fig. 1 B and C. The parenchyma cells in this region are usually packed with starch. When the leaf is older still this feature is lost. The pith cells collapse and break down and the whole base of the leaf becomes somewhat compressed.

When a leaf has ceased to grow it may remain green and healthy for some months but finally it withers. The tip of the leaf or, roughly speaking, the distal 10 cm. is usually brown in all leaves over 100 cm. long, and as a rule the older the leaf the longer is the brown region of the lamina. By the time that a leaf is as old as leaf 13 in Text-fig. 2 the withering has probably proceeded right to the base and the chlorophyll of the leaf is destroyed. The rate of withering is more rapid in its final stages, but it depends to a considerable extent on weather conditions and the degree of exposure of the habitat of the plant. When the leaf is entirely brown the aerial part of the lamina usually rots away at the ground level. Text-fig. 1 C shows the thick masses of old leaf bases which are borne on the stock of an old shoot.

This description of the growing-point and the leaves round it holds good whatever the age of the shoot, as is shown in Text-fig. 1 B and C. The difference in age is represented only by the difference in length of *xy* in Text-fig. 1 B and C, and correspondingly by the number of old leaves borne on this region.

At any time therefore a shoot will show the following types of leaves:

Type A. These have so far been called "rudimentary". They are the leaves most newly formed by the stem apex, are not more than a few millimetres high, and do not include any cells which are elongating rapidly in a vertical direction. (Leaves 1-4 in Text-figs. 2 and 3.)

Type B. These leaves are older than type A, and enclose them. They all have both meristematic and extending cells, and at any particular time they all have the same growth-rate. (Leaves 5-9 in Text-fig. 2.)

Type C. These are older than type B and are losing or have lost their meristematic activity. Hence their growth-rate soon falls away from that of the type B leaves and finally ceases altogether. They can thus be only slightly taller than the tallest type B leaves, and they may be shorter, since the brown part of the apex often becomes broken off. (Leaves 10-12 in Text-fig. 2.)

Type D. These are the oldest leaves of the shoot and include all those in which withering has extended throughout their length. (Leaves 13 and 14 in Text-fig. 2.)

This classification of the leaf types will constantly be referred to in discussing other work on the plant.

The uniform rate of growth of the type B leaves is remarkable, and it would be interesting to know whether any other plants show the same thing. The close aggregation of the young leaves immediately around the growing-point and the change in the type of growth which occurs when the leaf base has been pushed to a certain distance from the centre, suggests that there is a factor which dominates the growth-rate and acts within a space which has fairly definite lateral limits. It would also be possible to suggest that the meristem of each leaf, being derived from the same meristem originally, will react in the same way and to the same extent, to external influences; and further, that it will automatically become less active after a certain length of time. But there may be a difference of months between the time of origin of leaf 5 and leaf 9 in Text-fig. 2, and it is difficult to believe that the meristems of the two leaves will behave quantitatively alike without some co-ordinating influence.

IV. ANATOMICAL FEATURES, WITH SPECIAL REFERENCE TO THE AIR-SPACE SYSTEM AND THE MERISTEMATIC REGIONS

(a) Rhizome and stock

The rhizome has a diameter of 4-7 mm., the stele having a diameter about two-thirds of the whole. The stele is made very tough by the numerous bundles it contains and by a thick band of fibres, several cells deep, which forms the outer layer. The bundles have the phloem surrounded by xylem, and each bundle is enclosed in a ring of fibres. The ground tissue consists of rather thick-walled, but not lignified, parenchyma, packed with starch. The cells are rounded, leaving occasional small air spaces at the corners. Except for the outermost layers, the rhizome cortex when it is first differentiated consists of slightly stellate cells, between which there are abundant air spaces. This tissue also forms the cortex of the stock and is shown at *e* in Pl. II, fig. 3. The small circles such as that shown at *g* in the same figure are the areas of contact between one cell and another. In the rhizome this tissue breaks down at a distance of 1 cm. or less behind the apical bud, and forms a loose spongy mass which appears brown and dry. The limiting layers of the cortex are compact and consist of thick-walled cells. The rhizome bears scale leaves at intervals of about 7 mm. These are from 1 to 2 cm. long and almost cover the outer surface of the rhizome.

The stock is thicker than the rhizome, its diameter sometimes reaching 2 cm., but its tissues are very similar. The stellate cells of the cortex do not, however, break down as happens in the rhizome, but remain in the condition shown in Pl. II, fig. 3 *e*. The outermost layer of the fibres which separate the stele from the cortex has more abundant starch grains than the others, and in cross-section appears very like the endodermis of the root. It will be referred to as the starch sheath. In the uppermost part of the stock the starch sheath is not yet differentiated and the tissues of the stele are continuous laterally with those of the cortex, so that there is a possible communication between the air spaces of the two regions.

The stock is, of course, only the vertical continuation of the rhizome, so that the stele and cortex of the two are quite continuous. They are also continuous with those of the new rhizome buds which grow out from the base of the stock.

In the rhizome and stock, as well as in most other parts of the plant, tannin sacs occur. Plowman (1906) has given a list of Cyperaceae in which these cells occur, in his Tables I and II, pp. 12 and 16.

These tables show that while numerous species have a few tannin cells, not many possess them in abundance. In *Cladium Mariscus* the cells are rounded or oval and vary in size and shape. Their walls are not conspicuously thickened, and in unstained sections they are usually colourless, though occasionally yellow or brown. That their contents are of the nature of tannins is confirmed by the deep brown colour produced by warming gently with potassium bichromate solution. They take up methylene blue strongly, and after fixation and treatment with gossypimine and picric aniline blue they are brown, but the appearance of the cell contents varies considerably, as may be seen from Text-figs. 8 D, 14 and Pl. II, figs. 2 and 3. It seems as though the contents were liquid in the fresh state but are precipitated sometimes as granules, sometimes as a thin layer inside the cell wall, or else as a homogeneous mass.

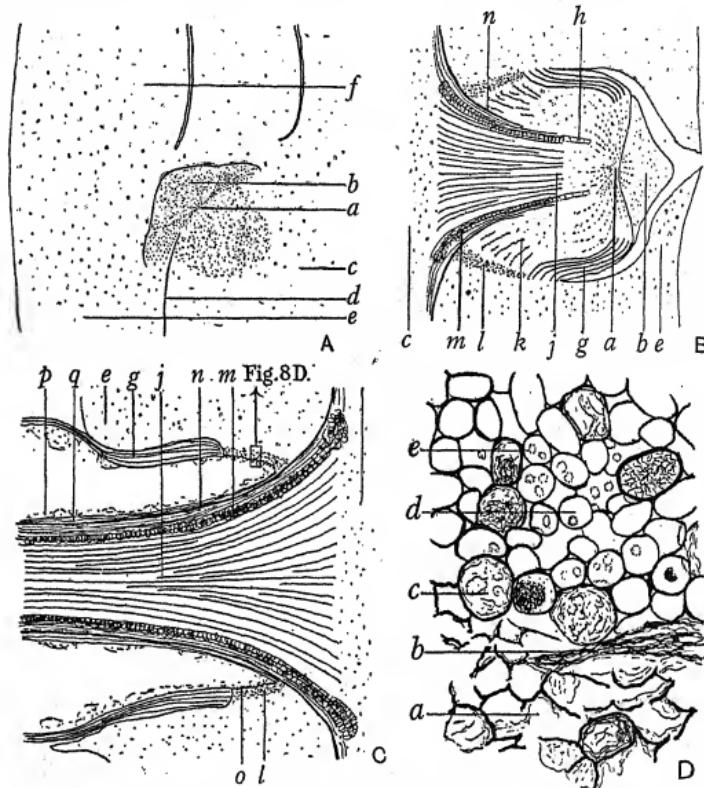
Tannin cells occur less frequently in the medulla of the rhizome than elsewhere in the plant. They are abundant in both medulla and cortex of the stock. The only types of tissue in which tannin cells have never been found are the epidermis and the groups of fibres.

(b) Roots

The primary roots are at present unknown; the others are produced adventitiously from the stock.

Text-fig. 8 A shows the earliest stage in root formation which has been found. This lies close to the growing point, just below the bases of the type C leaves (green, but no longer actively growing). The root initial is probably derived from a group of cells of the apical meristem which remain active longer than those round them, but it cannot be recognised until the latter have differentiated, giving the appearance of Text-fig. 8 A. The root cap is already distinct, and there is a clear separation of the meristem forming the cortex from that which forms the stele. Further, the endodermis of the root, when it differentiates, is continuous with the starch sheath of the stock. Hence the cortex of the root is continuous at the base with the cortex of the stock. Text-fig. 8 B illustrates a somewhat later stage in which differentiation has occurred at the base. There are fibrous cells on the outer surface of the root and in the centre of the stele (*g* and *j* in Text-fig. 8 B). Vascular bundles are developing in the outer layers of the stele, the earliest stages being seen at *h*, and the cortex is becoming loose and aerenchymatous. The starch sheath of the stock can be clearly seen continuing up into the root as the endodermis. At *l* there is a band of cells more closely packed than those on either side, but

they are rounded and show small intercellular spaces. This dense layer is precisely similar to the layer of cells at the base of the type C



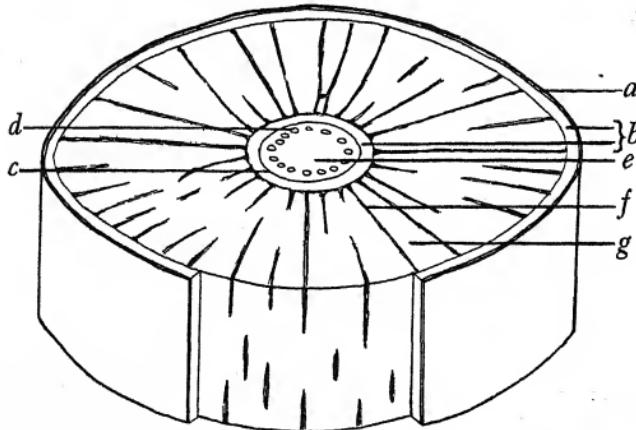
Text-fig. 8. A, B and C, longitudinal sections of the stock cortex to show stages in root development. *a*, growing point; *b*, root cap; *c*, stipe, *d*, starch sheath, *e*, cortex of stock; *f*, base of type C leaf; *g*, thick-walled cells of root surface; *h*, vascular bundles beginning to differentiate; *j*, fibres of root stock; *k*, root cortex just showing air spaces; *l*, more compact zone; *m*, vascular bundle; *n*, root endodermis; *o*, collapsed cells at base of root cortex; *p*, remnants of loose cortical cells; *q*, thick-walled cells of inner cortex. D, detailed drawing of area indicated in C. *a*, broken cells of root cortex; *b*, collapsed cells; *c*, tannin cell; *d*, rounded cells forming compact layer (cf. *l* in C); *e*, ring marking area of contact with the cell beneath.

leaf (see Text-fig. 2 c) and has a parallel origin, namely from the basal layers of a meristem.

The base of an older root is shown in Text-fig. 8 C. The cortex has broken down entirely; the cells at *k* in Text-fig. 8 B have collapsed,

while those just behind take a lignin stain. The region indicated at *l* in Text-fig. 8 C is shown in detail in Text-fig. 8 D. The cells are more or less rounded but not in contact at every point. The rings indicated for instance at *e* in Text-fig. 8 D mark the area of contact between one cell and another.

The structure of the mature root resembles that of the root of many aquatic Monocotyledons and is illustrated in Text-fig. 9. It shows the narrow polyarch stele whose centre consists entirely of fibres, the broad cortex bounded both inside and outside by two or



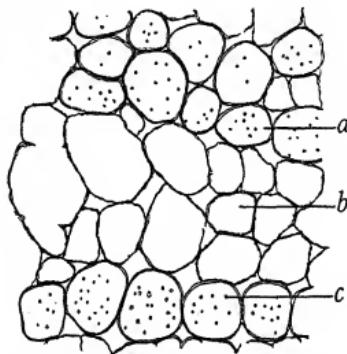
Text-fig. 9. Diagram to illustrate the root structure. The outer layers are shown partly cut away. *a*, exodermis; *b*, thick-walled layers of cortex; *c*, endodermis; *d*, vessels; *e*, fibres; *f*, radial supporting strands; *g*, loose cortical parenchyma.

three layers of thick-walled cells and the thick-walled exodermis on the outside. The central region of the cortex in the young stage consists of thin-walled parenchyma supported by bands of cells with slightly thicker walls showing definite pits. These bands of cells lie radially and some stretch right across the cortex, but others are attached either to the stele or to the peripheral layers, while some are entirely isolated. They do not extend far in the longitudinal direction as can be seen in Text-fig. 9, where the peripheral layers have been partly cut away. The parenchyma cells are rounded and show air spaces in the corners between them; the walls are very brittle and break down almost as soon as they are fully differentiated,

so that the cortex becomes a large air space supported by the girder-like bands of resistant cells with the parenchyma forming a spongy mass of shrivelled cells.

Text-fig. 10 shows the appearance of a small part of the root cortex in transverse section where the parenchyma is just starting to break down between the two bands of resistant cells. The lower band is only one cell in width, the upper band two or three.

The root structure just described is characteristic of plants which grow in the ditches and shallow ponds which are found at Wicken Fen. Their roots grow in peat which is always entirely waterlogged and may be covered by as much as a foot of water in winter. Branch

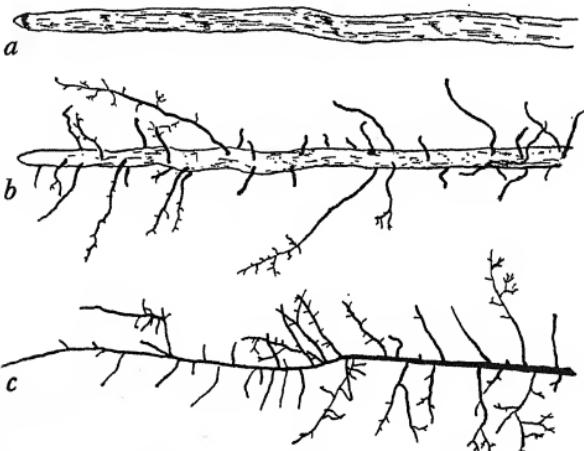


Text-fig. 10. Part of root cortex in section. *a*, cell of radial supporting band; *b*, thin-walled parenchyma cell; *c*, pit in cell wall.

roots are infrequent, and the fat fleshy appearance of the root is shown in Text-fig. 11 *a*.

When the plants are growing in the slightly higher ground which is occupied by the Mixed Sedge (*Cladio-Molinietum*) and which is not always waterlogged, the roots differ in that they produce many more lateral branches, shown in Text-fig. 11 *b*, and also in Text-fig. 11 *C*, which illustrates a plant from the *Cladio-Molinietum*. The main roots are still, however, somewhat fleshy. Occasionally plants are found whose root system has become exposed to the air, and here the roots are not fleshy but break up into fine branches with very numerous fine laterals as in Text-fig. 11 *c*. The whole root system forms a dense mat round the base of the plant. The same type of root system is formed by plants grown under experimental conditions in the surface water of shallow tanks where the water is nearly, if not quite, saturated with oxygen.

Cladium Mariscus can thus be added to the list of plants in which the type of root system varies considerably with the oxygen concentration of the surrounding medium. Its behaviour may be compared with that of *Phragmites communis* described by Weaver and Himmel (1930). They found that plants of *Phragmites*, when grown in waterlogged and poorly aerated soil, produced two types of roots, a finely branched surface root system, and coarser, downwardly directed unbranched roots.



Text-fig. 11. Variation in type of root produced in different conditions. *a*, in submerged peat; *b*, in damp peat; *c*, in surface layers of peat or in well-aerated water.

(c) Leaves

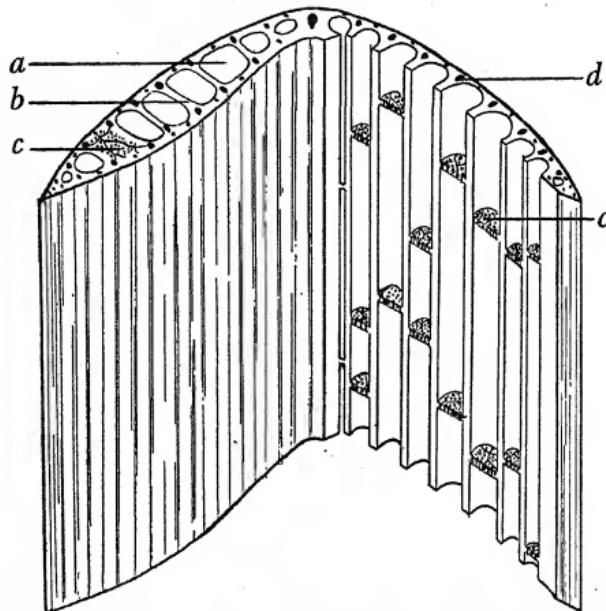
(i) Tissues of the fully differentiated part of the lamina.

The anatomy of the leaf varies along its length, but the variation is mainly quantitative. Most of the types of tissue are found in all parts of the leaf, and Text-figs. 12-15 illustrate their disposition about halfway up a fully grown leaf, i.e. 70-80 cm. above the base.

Text-fig. 12 is a diagram to illustrate the leaf structure at this level.

The central spaces do not form unbroken channels throughout the length of the leaf, for they are traversed at intervals of a few millimetres by horizontal septa, one of which is indicated at *c* in Text-fig. 12. The leaf is thus divided up into compartments as is

seen also, for instance, in *Typha* and *Sparganium* spp. Vascular bundles are distributed with some regularity but are not absolutely constant. With respect to the orientation of the bundles, the leaf is isobilateral, so that in every bundle the xylem is towards the interior of the leaf. There is usually a single bundle in the keel of the leaf, but it is not always symmetrically placed in the median line. Text-

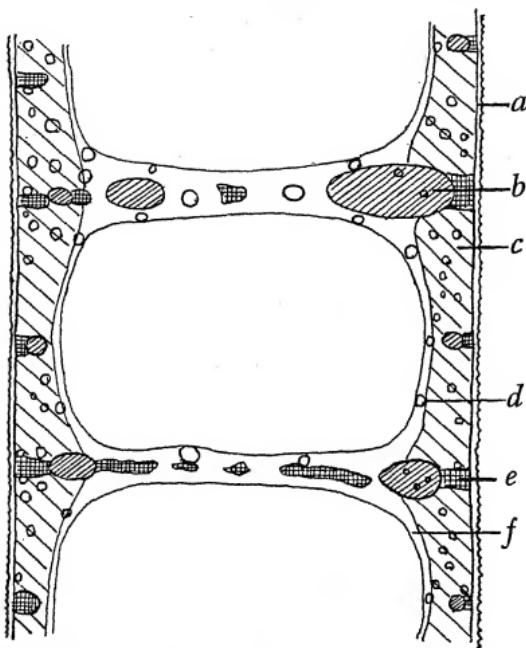


Text-fig. 12. Diagram to show leaf structure at about 70 cm. from the base.
The front surface of half the leaf has been cut away. *a*, air space;
b, vertical septum; *c*, horizontal septum; *d*, vascular bundle.

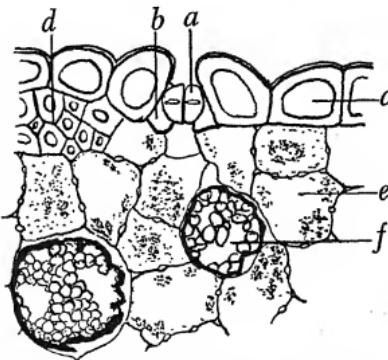
fig. 13 shows a part of a transverse section on a larger scale to illustrate the distribution of the various types of tissue.

Mechanical tissues are abundant. Not only are there numerous isolated strands of fibres, but each vascular bundle is encircled by a ring of them and a band of fibres separates the phloem from the xylem.

The epidermal cells are thick-walled and rectangular, elongated in the length of the leaf, and the cuticle is thick. Stomata occur on both sides of the leaf, set with their long axes in the length of the leaf. They are not found overlying the bands of fibres. Their structure



Text-fig. 13. Part of the transverse section corresponding to Text-fig. 12, enlarged to show the distribution of the tissues. *a*, epidermis; *b*, vascular bundle; *c*, chlorophyllous palisade; *d*, tannin cell; *e*, fibres; *f*, parenchyma.

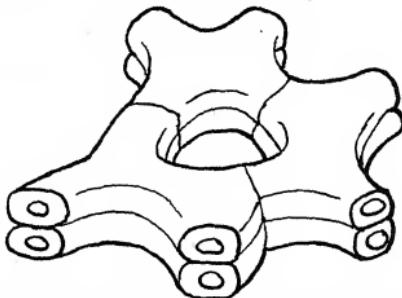


Text-fig. 14. Transverse section of a stoma and adjacent cells. *a*, guard cell; *b*, subsidiary cell; *c*, epidermis; *d*, subepidermal fibres; *e*, chlorophyllous tissue; *f*, tannin cell.

is of a type common in the Cyperaceae, with dumb-bell-shaped guard cells subtended by narrow subsidiary cells which run parallel to the length of the pore. The adjacent epidermal cells overarch the stoma slightly. Text-fig. 14 shows a stoma in cross-section.

The chlorophyllous tissue forms a compact layer underlying the epidermis. The walls of the cells are not in contact at every point, but many small air spaces are present between them which give the walls a beaded appearance in section as shown in Text-fig. 14. This feature can be made out in both fresh and fixed material.

The cells bordering the central spaces and forming the ground-work of the longitudinal septa are larger than the assimilatory cells,

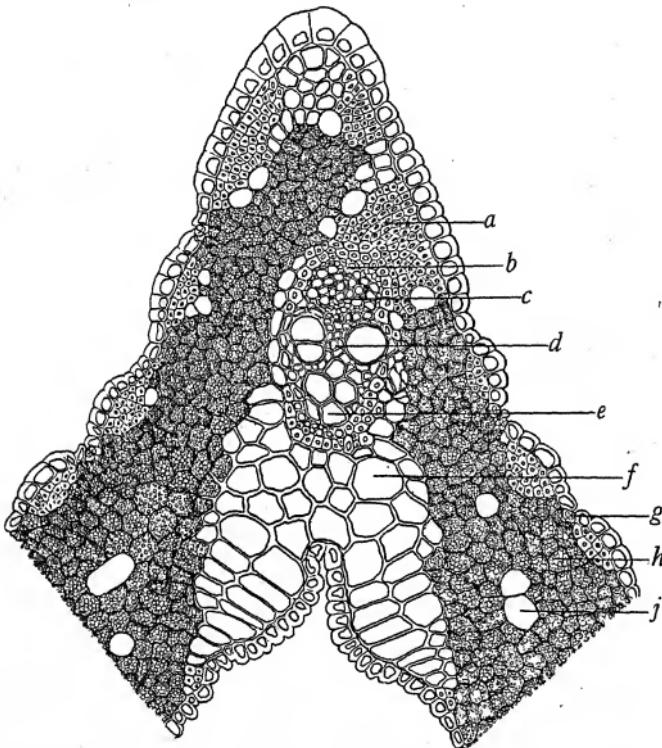


Text-fig. 15. Diagram to show the type of cell which forms the ground tissue of the horizontal septum.

and the walls are slightly thickened. Tannin cells are very frequent as may be seen from Text-fig. 13.

The horizontal septa are not continuous plates of tissue but a network formed by stellate cells, supported by small bundles which are given off from the main longitudinal bundles of the leaf, and lie in the plane of the septum, branching occasionally. The stellate cells are flattened in the plane of the septum, and two or three tiers of cells overlie each other as is indicated diagrammatically in Text-fig. 15. The walls are thickly and rather irregularly lignified. Adhering to the septum there can usually be seen the remains of the thin-walled cells whose collapse has left the large central spaces in the leaf. The morphology and development of diaphragms of water plants in general have been discussed by Snow (1914). At the level of the leaf at which Text-fig. 12 was taken the cells at the keel of the leaf are not noticeably different from the other parenchymatous cells, but higher up there are specialised cells at the inner side of the angle of

the leaf, corresponding in position to the hinge cells of certain grasses. When a leaf is dried the two halves of the leaf fold together, and it seemed possible that this might be due to the same type of hinge mechanism as those grasses show. This, however, is not the case, for



Text-fig. 16. Transverse section across the keel of a leaf at about 100 cm. above the base. *a*, fibres; *b*, protophloem; *c*, phloem; *d*, xylem; *e*, protophloem; *f*, thick-walled keel cells; *g*, stoma; *h*, chlorophyllous palisade; *j*, tanin cell.

the cells are thick-walled as Text-fig. 16 shows. They are not lignified and give a weak cellulose reaction with Schulze's solution. If a dry section of a wilted leaf be irrigated with water the two halves can be seen to expand, and the angle between them widens with very little change in shape or position of the cells at the keel itself. The reason

for the folding seems to be that there is a slightly larger proportion of thin-walled cells along the inner sides of the leaf than on the outside, so that the shrinkage on drying is greater in the former.

The more distal region of the leaf differs from the middle region in the following points as well:

(1) Sharp spines occur on the keel and margins. They are upwardly directed with a decurrent base and give the leaf its characteristic saw edge. The free part may be up to 0·3 mm. in length. In section they resemble stone cells and are lignified.

(2) There is a smaller proportion of thin-walled tissue relative to other types. The subepidermal fibres are more numerous, and the leaf has such a small thickness that the main bundles of the two surfaces often meet in the middle of the leaf.

(3) There is a loose central pith in each "compartment"; there are, however, numerous air spaces between the cells.

The lower part of the leaf, on the other hand, has a much smaller proportion of mechanical tissues. The groups of fibres are much smaller relative to the size of the cross-section, are not so thick-walled, and are almost absent in the longitudinal septa. The epidermal cells have very much thinner walls.

(ii) *The basal part of the lamina and its attachment to the stock.*

The lowest 1 cm. of the leaves is of particular interest in relation to their growth and differentiation and to the possible continuity of the air spaces of the leaves with those of the stock.

The rudimentary (type A) leaves show nothing but closely packed meristematic cells. The type B leaves show meristematic cells for a distance of 1 cm., at any rate in the winter; the distance may be longer in the summer, but this is not yet known for certain. These leaves, however, all show one or more vascular strands running through the meristem and passing down into the stock. In Text-figs. 3 and 6 the position of those bundles which already show protoxylem and protophloem is indicated for all the leaves up to no. 7; the leaves outside this show well-developed xylem in all the bundles of the abaxial series. These strands in the early stages have one or two spirally thickened tracheids (protoxylem) and two or three elongated protophloem cells. The vascular tissues which will develop later are foreshadowed in transverse section, for the cells which will form them are rather smaller in area than the rest.

The way in which the provascular tissues link up with the bundles in the stock is very variable, and it is impossible to state any rule

or present any idealised diagram of their course. Some idea of the general type of arrangement may be obtained from Text-fig. 2. The bundles from younger leaves on the whole tend to cross those from older leaves and run down the stock more marginally. In the meristematic part of the stock procambial strands can be seen passing almost horizontally from the centre to the more peripheral region. Here they turn downwards and join the older bundles, usually on their outer side.

Now since leaves such as 5, 6 and 7 in Text-fig. 2 are fully differentiated in their upper parts there must be numerous xylem strands in those leaves which have no connection with the vascular system of the stock. All the adaxial bundles and some of the abaxial bundles are in this condition. On the other hand, it will be seen that no leaf which has rapidly extending or differentiating cells (i.e. no type B leaf) is entirely without vascular connection with the stock. One might in fact conclude that the formation of a provascular supply for any leaf is a necessary condition for that leaf to grow upward at an appreciable rate.

There is a regular order in which these provascular strands differentiate in the leaf base. Text-fig. 3 B, leaf 5, shows the first stage with only one protoxylem strand lignified, that in the keel of the leaf. The next two to be lignified are roughly at the mid points of the arms of the V, then those on the side farther from the keel (Text-fig. 3 B, leaf 6), and then, in a less regular order, the remainder of the strands in the abaxial series. The adaxial bundles at the leaf base do not show any lignification until the leaf is ceasing to grow, i.e. until they become type C leaves. At this time also all the fibres and xylem elements at the base of the leaf become lignified. Leaf 11 of Text-fig. 2 would have reached this state.

In type B leaves tannin cells can be seen at a distance of 1 mm. from the base in association with the vascular strands. The horizontal septa appear as bands of flattened cells crossing the leaf at a distance of only 2 mm. from the base. At this stage they are close together, say 0.3 mm., so that the distance between them increases considerably before they are finally differentiated.

Intercellular air spaces do not occur in the type A leaves, but in type B the air spaces which occupy such a large volume in the differentiated part of the leaf are continued down to the base of the leaves as very narrow passages between the vertical rows of meristematic cells. The stock meristem when seen in transverse section also shows small air spaces so that there is no complete barrier to

the passage of gases from the upper parts of the type B leaves to the tissues of the stock. On the other hand, the freedom of movement of gases across the considerable length of meristem must be very limited compared to the freedom of movement down a corresponding length of the differentiated part of the leaf.

In a leaf which is just becoming mature, e.g. no. 10 in Text-fig. 2, the pith cells have differentiated almost to the base of the leaf and the cells are much more loosely packed, with a correspondingly larger proportion of air space. The region at *c* in Text-fig. 2 is shown in detail in Pl. II, fig. 1. The rather more densely packed cells at *b* in Pl. II, fig. 1 are the last cells to be meristematic, and they will soon differentiate into pith cells like those above them at *a*. The cells at *c* are slightly flattened, and below them at *d* are the round cells of the stock cortex, with numerous tannin cells. The flattened cells do not form an unbroken layer of cell tissue but show small spaces as is natural, since there were spaces in the meristem from which all this region is derived.

Pl. II, fig. 2, shows a later stage, in a leaf such as no. 12 in Text-fig. 2. The pith cells (*a*) are breaking down: the flattened cells (*c*) have become compressed and torn. Their walls turn yellow, but they do not give any obvious reaction with Sudan III, which indicates that they are not much suberised. Nor do they react to lignin stains. Immediately below these cells, however, one or two layers (*d*) may show a slight lignin reaction in their walls. As the leaf grows older, the lignification continues deeper into the cortex until, as Pl. II, fig. 3 shows, there is a strong band formed right across the base of a leaf. The walls of the lignified cells are not greatly thickened, and small slit-like pits can be seen on them. It is possible to see occasional air spaces between the cells as might be expected, since the lignification occurs in cells of the cortex which were previously rounded and loosely packed. By the time that the leaf base has reached this state the upper part is entirely brown and withered. The changes described cannot be compared with the formation of a true absciss layer, for there is no formation of a secondary meristem, the vascular bundles are not severed and the leaf can never be pulled off to leave a clean scar.

It follows from this process of differentiation that the volume of relatively compact tissue which separates the large air spaces of the leaf from the loose aerenchymatous tissue of the stock cortex is very much smaller in type D leaves than in type B.

V. CONCLUSIONS AND SUMMARY

Cladum Mariscus is an evergreen geophytic Monocotyledon which has been studied as it grows at Wicken Fen, Cambs. Until it flowers, only the leaves appear above ground, and all the growing parts of the plant are buried in several inches of soil or water. The leaves are borne on a short upright stock, and vegetative reproduction is carried out by horizontally creeping rhizomes.

The meristematic regions are confined to the root tips and to the leaf bases in the centre of the shoot, with a narrow region of the stock below these bases. The form of the mature leaves is readily explicable in terms of the changing shape of the basal meristem as the leaf develops. Every leaf passes through exactly the same life cycle as the one before it, quite independently of the season, the action of which is only to accelerate or retard morphological change, but not to direct it. The history of each leaf is divided into four well-defined phases: the first where it consists entirely of meristematic cells, but is still rudimentary and not actively growing (type A), the second where it is actively growing and differentiating (type B), the third where its growth soon ceases to be active, but where it is still green (type C), the fourth where it is brown and withered throughout its length (type D). All the type B leaves grow at the same rate, and this common rate is a feature in the plant's behaviour which lends itself to observation and suggests points of physiological and ecological interest, for it is very sensitive to external conditions.

Like most aquatic plants, this species is characterised by the large volume of internal air space. The proportion of air space to cell volume varies considerably in different parts of the plant. It is largest in the leaves: they show longitudinal channels which are empty or only contain very loosely packed pith cells, and are traversed at intervals by perforated septa. The ground tissue in the cortex of the root breaks down as does also that of the rhizome, so that the stele in both cases is surrounded by tissue very largely consisting of air space. The cortex of the stock is also aerenchymatous, as the cells are slightly stellate; the stele of the stock and rhizome had small intercellular air spaces which probably link up with one another to form a continuous system. There are, however, important zones of tissue where the air-space system is very much restricted, that is, in the meristematic regions at the base of growing leaves, across the transition from leaf to stock at the base of old leaves, and the transition from stock cortex to root cortex. In none of these

zones, however, are air spaces entirely absent, so that although this cannot be proved by anatomical evidence, the probability is that all the air spaces of the plant form one continuous system. The width of the zone where the air space is restricted is not great (only four or five cell layers) in the case of the transition from root cortex to stock cortex; it is of the same order for the transition from leaf to stock at the base of the older leaves, but it is much greater for the leaves which have a meristem at the base. For gas to pass from a growing leaf into the cortex of the stock it would have to filter gradually downwards through a centimetre or more of leaf meristem and then outwards through the cells of the stock apex which are only just starting to differentiate into a more or less aerenchymatous tissue. Hence it is only the oldest green leaves, and the dead brown ones, that afford a relatively free diffusion path to the internal air-space system of the underground parts.

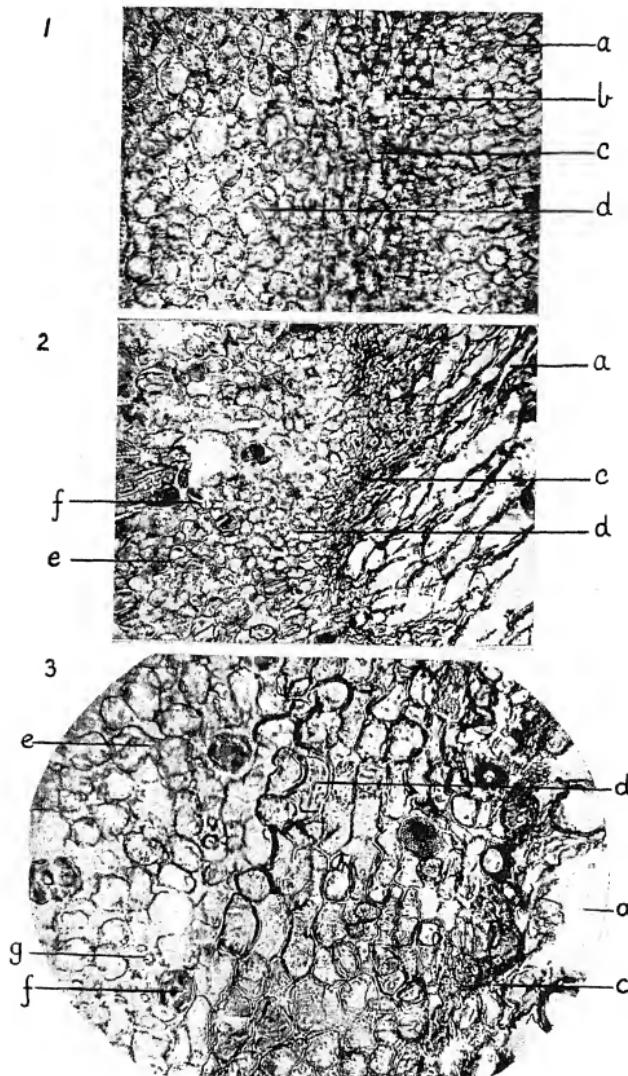
The writer is very greatly indebted to Dr H. Godwin for his encouragement and suggestive criticism, both in the course of the research and in the preparation of this paper.

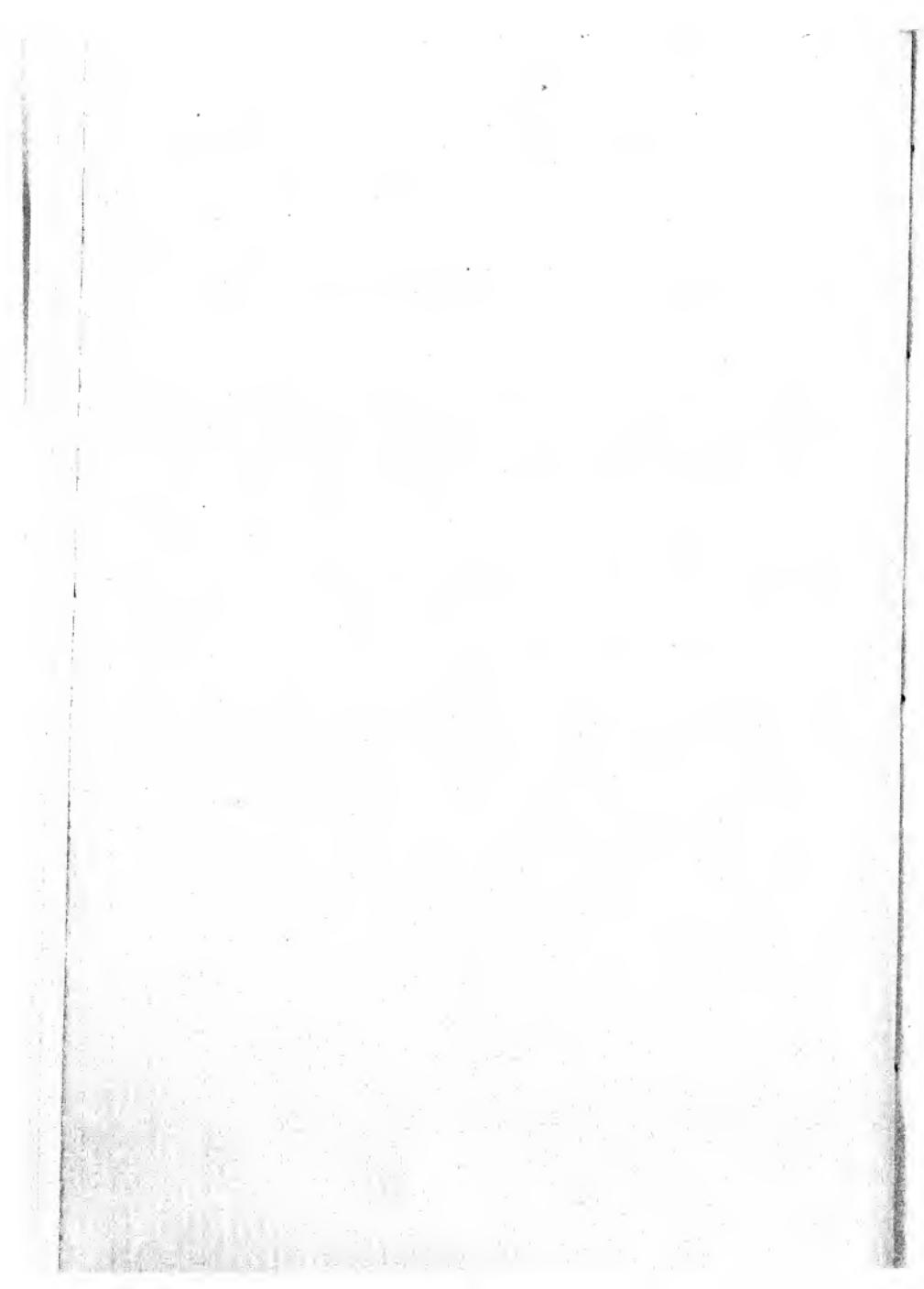
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EXPLANATION OF PLATE II

Figs. 1, 2 and 3. Details of the regions indicated in Text-fig. 2 to show the changes which take place at the base of the leaf as it grows older. *a*, pith of leaf; *b*, basal cells of leaf meristem; *c*, slightly flattened cells which become crushed, as in Figs. 2 and 3; *d*, layers of cells which become lignified as shown in Fig. 3; *e*, deeper lying layers of stock cortex; *f*, tannin cell; *g*, area of contact between one cortical cell and another.

CONWAY—*CLADIUM MARISCUS*



AUXIN AND CORRELATIVE INHIBITION

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(With 2 figures in the text)

INTRODUCTION

AUXILLARY buds of seedlings of Leguminosae are inhibited in nature by a hormone produced from the young leaves of the apex (Snow, 1925, 1929 *a, b*). Recent work of Thimann and Skoog (1933, 1934 *a, b*) shows that similar inhibition occurs when the plant hormone auxin is applied to the top of a decapitated pea seedling. Laibach also (1933 *a*) has shown that pollinia of orchids, which secrete an auxin, inhibit the axillary buds when applied to the top of a decapitated bean stem. Previously the chief known characteristic of auxin had been its power to accelerate growth: thus the work of Thimann and Skoog and of Laibach, since confirmed by Muller (1935) and by Uhrova (1934) demonstrates a situation where a single hormone at once inhibits and accelerates plant growth. To account for this double effect, two theories have been put forward; Thimann and Skoog's "direct theory" (1933, 1934 *a, b*) and Laibach's "indirect theory" (1933).

The direct theory supposes that the arrival at the lateral bud of auxin from an external source prevents the lateral bud from synthesising auxin, and further that the bud is unable to elongate without auxin of its own manufacture. One obvious objection to the theory is the absence of any explanation as to why, if auxin is present in the bud, it is not used for bud elongation. A second difficulty is connected with the movement of auxin in the plant. Most workers (for instance van der Weij, 1932; Thimann and Skoog, 1934 *a*; Mai, 1934) are agreed that the travel of auxin in stems and coleoptiles is strictly polar; it will only travel in a morphologically downwards direction. At the same time Snow (1929 *b*, 1931) has shown that inhibition can travel upwards for a considerable distance with undiminished intensity. Experiments to be reported hereafter suggest that auxin may travel a short distance up the stem, but unless we allow with Thimann and Skoog a leakage of auxin from a polar tissue of the stem into some non-polar part, any long-distance travel up-

wards is difficult to reconcile with the fact of auxin's mainly downward transport.

The second theory (Laibach, 1933) supposes that inhibition is not due to a direct action of the auxin on the lateral buds. He and others (Mai, 1934; Muller, 1935) believe that the auxin first takes part in some growth process in the main stem and that this growth then secondarily inhibits the bud. This theory is essentially the same as was put forward by Loeb (1924, pp. 101 and 108) and discussed by Snow (1932, p. 103).

MATERIAL AND METHODS

The experimental plant was *Pisum sativum*, race Thomas Laxton, grown in a mixture of 2 parts sawdust to 1 part sand in the greenhouse. This plant and method had been found satisfactory in producing plants of very similar age and condition. The hormone used, unless otherwise stated, was pure synthetic hetero-auxin (β -indolyl acetic acid), which, so far as is at present known, exerts in all respects the same physiological effects as natural auxin. The hetero-auxin-lanoline paste used was obtained by stirring small quantities of a solution of known strength into anhydrous wool fat until it would take up no more solution, in the manner devised by Laibach. For convenience the leaves of the plant will be numbered in ascending order from the bottom of the seedling: thus the two first-scale leaves will be Nos. 1 and 2, and the first complete leaf No. 3. The internodes will be numbered from the leaf below them: thus the internode above the lowest complete leaf will be internode No. 3.

EXPERIMENTS

(i) Inhibition and the position of the source of auxin

It seemed of interest to decide whether the two opposing effects of auxin on the plant, its growth inhibition and growth acceleration, were due to the fact that in one case the auxin was travelling up the plant and in the other down. For in experiments where stem growth has been accelerated it has always been travelling down the plant, while in the inhibition of shoots it has always been coming from a position morphologically below the inhibited part. In bud inhibition also the effect must travel upwards, since the bud is of necessity approached from its basal end. The following experiments are an attempt to supply auxin to buds and shoots in such a manner that it approaches them from the base only.

(a) *Buds and shoots in auxin solution.*

Exp. 1. Cuttings were taken from pea plants by cutting the stem 4 cm. below and 2 cm. above a leaf (Fig. 1). These pea plants had been carefully matched in twos or threes as the experiment required, but the pairs might vary by half a plastochron among themselves. The cuttings were placed in specimen tubes, one cutting per tube, with the long end of stem dipping into solution. Solutions were renewed every 2 days from freshly made stock: a few drops of a strong thymol solution were added to lessen the danger of bacterial contamination. About 0.5 c.c. solution was taken up daily by each cutting. In an experiment using leaf 5 from a plant with five leaves fully extended, the buds originally 1 mm. in length reached the following lengths in mm. after 8 days (Table I):

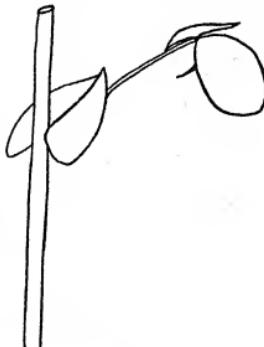


Fig. 1. Cutting.

TABLE I

Water control	4 in 10^{-6} auxin solution	2 in 10^{-6} auxin solution
11, 9, 7, 7, 4, 6	1, 1, 1, 1, 1.5, 1	2, 2.5, 1.5, 2, 4, 4

Thus auxin in such weak solution as 2 or 4 in 10^{-6} caused strong inhibition of axillary buds.

Exp. 2. An experiment similar to the above was set up, using whole shoots instead of cuttings. The shoots were obtained by cutting the stem below the lowest leaf. The length of the youngest internode and any younger internode that might develop during the experiment was measured daily. An experiment was set up on 9 March with shoots from plants that had five leaves fully expanded, paired in experiment and control. In the shoots with the stronger hetero-auxin solution, the length of internode 5 varied at the beginning of the experiment from 5 to 10 mm., and in those with the weaker solution from 2 to 5 mm. The following (Table II) are the average length of internode 5 after 6 and 8 days, and the average length of internode 6, which developed during the experiment, after 8 and 10 days. There were six shoots in each group. The two groups of shoots in the two different hetero-auxin solutions were each paired with a separate group of controls.

TABLE II. *Lengths of internodes in mm. after various times*

	1 in 10^6 auxin solution			5 in 10^6 auxin solution			
Control	Av.	solution	Av.	Control	Av.	solution	Av.
Internode 5 after 6 days:							
27, 20, 29	22, 21, 23	22·4	23, 21, 21	20·8	14, 19, 21	16·4	
23, 25, 24	21, 25, —	—	20, 25, 15	—	17, 13, 14	—	
Internode 5 after 8 days:							
29, 31, 33	31·0	30, 32, 28	29·8	26, 26, 29	30·8	28, 30, 31	29·3
32, 34, 28	—	30, 29, —	—	35, 33, 36	—	30, 26, 31	—
Internode 6 after 8 days:							
21, 32, 25	27·5	17, 18, 25	19·8	18, 24, 24	20·8	14, 7, 17	12·8
25, 30, 32	—	21, 18, —	—	21, 19, 22	—	14, 9, 16	—
Internode 6 after 10 days:							
35, 40, 43	41·5	32, 30, 30	30·2	37, 38, 28	34·0	30, 31, 26	25·3
45, 40, 46	—	29, 30, —	—	31, 34, 35	—	22, 17, 26	—

It would appear from the above experiment that the growth of a young internode, subjected to a weak hetero-auxin solution, is partially inhibited for some time, but finally reaches nearly the normal length, since internode 5, which was young at the beginning of the experiment, after 4 days has a ratio of 1·64/2·08 for experiment/control, but after 10 days 2·93/3·0. The growth of a still younger internode, in this case internode 6, that had been supplied with hetero-auxin even earlier, was not only inhibited at first, but also failed to reach the normal final length, since the experiment/control ratios in the two experiments after 10 days were 3·02/4·15 and 2·53/3·4. As shown above, the final lengths of internodes of about 5 mm. length or more at the beginning of an experiment appeared to be unaffected by the hetero-auxin solution.

Exp. 3. The following is an experiment with stronger hetero-auxin solution. In this experiment a shorter length of shoot was used from plants which had eight leaves fully developed. The shoot consisted of internode 6 and all growth above it. The shoots were matched in threes: internode 8 varied in length from 4 to 10 mm. at the beginning of the experiment. The experiment was set up on 25 March and internode 8 measured every second day (Table III).

TABLE III. *Average length of internode 8 in mm. in five plants*

	Number of days after operation				
	2	4	6	8	10
Water control	16·4	30·0	42·8	46·2	48·8
2·5 in 10^6 hetero-auxin solution	14·8	19·0	27·2	30·4	31·8
5 in 10^6 hetero-auxin solution	9·8	16·8	21·6	24·2	26·2

This experiment confirms the previous one in showing that growth of a young shoot is retarded by hetero-auxin solution supplied at the base. It also suggests that the final length of the internode is affected, as at the end of the experiment (4 April) the auxin plants seem to be reaching a final value lower than the controls.

The above experiments show that growth retardation by auxin is not a phenomenon peculiar to axillary buds; another part of the shoot, the elongating internode, can be affected. These experiments, however, do not distinguish between the direct and indirect theory of auxin action. The auxin, although it is supplied at the base of the shoot, is present in the plant water supply and can travel in the transpiration stream at least as far as the older elongating region where it could act directly on the parts concerned.

(b) *The effect of lanoline containing hetero-auxin on shoot elongation.*

The following variation of the foregoing experiments was undertaken with the object of preventing hetero-auxin from passing upwards in the transpiration stream: hetero-auxin was in this experiment applied to the plant in lanoline.

Exp. 4. An internode of a rooted and growing pea seedling was split longitudinally to within 0·5 cm. of the nodes above and below with a sharp-pointed scalpel. Lanoline containing hetero-auxin was inserted into the split and was spread round the outer surface of the split internode. Controls were treated similarly with pure lanoline. In a few plants the halves of the split internodes buckled, the hetero-auxin paste being very strong ($1 \text{ in } 10^4$), and in a few plants broke, but generally they remained in their original position. The plants in which one half broke were rejected.

Table IV shows the growth of young internodes (5) of plants treated in this way. The experiment was set up on 5 June with six pairs of plants with five fully developed leaves. The lanoline was applied to internode 4 and internode 5 was measured daily. Internode 5 measured from 3 to 5 mm. at the beginning of the experiment. The plants remained in good condition throughout the experiment except for same yellowing of the lower leaves in both experiment and control.

TABLE IV. *Average length in mm. of internode 5
in six pairs of plants*

	Number of days after operation								
	1	2	3	4	5	6	7	8	9
Pure lanoline control	10	19	28	45	49	52	53	53	53
$1 \text{ in } 10^4$ hetero-auxin in lanoline	10	13	16	22	24	25	27	27	27

It can be seen that internode 5 was not only retarded in growth but failed to reach a final length anything like as great as in the control plant. The total growth also was not as large in the experimental plants as in the control: for after 17 days the average length of the control plants was 24.1 cm. and of the experimental plants 11.7 cm. Some of the later internodes of the experimental plants were longer than internode 5, but they were not in any case as long as the control internodes.

An unexplained peculiarity of this experiment was the outgrowth in the experimental plants of the buds in the axil of leaves 2 and 3, below the hetero-auxin paste: 6 days from the commencement of the experiment they reached lengths between 4 and 6 mm. and were removed in order that the experimental plants should be comparable with the controls.

Weaker hetero-auxin pastes were also tested, and paste of 2 in 10^5 appeared to be nearly the weakest that would affect growth, as is shown by the following experiment.

Exp. 5. Plants with five leaves fully expanded were split in internode 4 on 6 July. Table V shows the length of internode 5 during the course of the experiment.

TABLE V. *Average length in mm. of internode 5 in seven plants*

	Number of days after operation					
	1	2	3	4	5	12
Pure lanoline control	6.5	10	15	28	35	39
2 in 10^5 hetero-auxin paste	6	8	12	19	29	38

It will be seen from Table V that weak hetero-auxin paste, of 2 in 10^5 , only affects the growth rate for a few days after its application, retarding it slightly, and has no effect on the final length of the node. Thus internode 5 gave an experiment/control ratio of 2.9/3.5 after 5 days, and the ratio after 11 days was 3.8/3.9. The experiment/control ratios of the total length of the plants were after 5 days 3.7/4.5 and after 11 days 9.4/9.6.

1 in 10^5 hetero-auxin paste had hardly any appreciable effect on the growth of a normal plant, but on plants whose young leaves were removed as they reached a length of 5 mm. it had a marked effect, as is shown by the following two experiments.

Exps. 6 and 7. Table VI gives the results of experiments set up in July, with plants that had fully expanded five leaves. Both these experiments were performed under identical external conditions.

TABLE VI. Average length in mm. of internode 5 in five plants with leaves removed

	Number of days after operation							
	1	2	4	5	6	7	10	
Pure lanoline control	12.6	22	41.6	44.4	45.2	46.2	48	
1 in 10^5 hetero-auxin paste	10.6	16.6	26.4	27.2	28.2	30	32	

Average length in mm. of internode 5 in five intact plants

	Number of days after operation						
	1	2	3	4	5	12	
Pure lanoline control	8	11.5	17	26	32	36	
1 in 10^5 hetero-auxin paste	8	10.6	14	24	28	32	

Thus 5 days from the beginning of the experiment, the ratio experiment/control for internode 5 in the experiment where the leaves had been removed is $2.72/4.44$ and in the experiment where the leaves remained $2.8/3.2$. Ten days from the beginning of the experiment the ratio experiment/control for internode 6 is $2.24/3.9$ where the leaves had been removed and $3.35/3.9$ where the leaves were untouched. The above experiment indicates that hetero-auxin applied from below has a greater inhibiting effect on a plant whose young leaves are removed: this agrees with Snow (1931) who found that the leaves of a shoot could protect it from its twin shoots inhibiting effect.

The results of the lanoline experiments confirm the results of the hetero-auxin solution experiment, and show that hetero-auxin supplied to a part of the plant morphologically below a growing internode will retard and inhibit the growth of that internode.

Although all authors are agreed that growth is accelerated by auxin travelling down the stem, it was thought advisable at this juncture to test the accelerating properties of the hetero-auxin lanoline paste used in the above experiments. As the elongation of a broad-bean stem that has reached manageable proportions seems almost independent of external auxin supplies (Thimann and Skoog, 1934 a) if grown in the light, the experiments were performed in the dark. The pea seedlings after being in the dark for 3 days were decapitated at the top of a very young internode, disbudded, and lanoline containing hetero-auxin applied to the top of the stem. $2 \text{ in } 10^5$ was thought a good strength to test on the assumption that at least as much and probably more hetero-auxin would pass into the plant than from the minimum inhibition strength ($1 \text{ in } 10^6$). This assumption was made on the ground that auxin is more likely to pass

down the plant than up and on the greater efficiency (accelerating (Thimann and Skoog) and inhibiting) of auxin in the dark.

After 7 days the youngest internodes, which had been 3 mm. long at the beginning of the experiment, had reached the following lengths (Table VII):

TABLE VII. *Length of young internodes in mm. after 7 days*

Pure lanoline control	24, 20, 19, 22, 22, 30, 26
Hetero-auxin paste	41, 38, 45, 44, 27, 45, 14

Thus hetero-auxin paste, of a strength capable of partially inhibiting the growth of young internodes when applied to a part of the stem morphologically below them, accelerates the growth of similar internodes when applied to their upper ends. Zimmerman and Wilcoxon (1935) also find that hetero-auxin when applied to the stem of a seedling, the whole way from tip to base, retards the growth of the seedling; but at the same time they do not make it clear that retardation of growth only occurs when auxin is placed in a position morphologically basal to the region of elongation.

The inhibition results with hetero-auxin in lanoline paste could be explained either on the direct or indirect theory of auxin action: for supporters of the direct theory could suppose that the auxin can diffuse laterally into the upward-moving transpiration stream. Some earlier experiments carried out with cuttings are therefore of interest here. They were set up in an attempt to admit auxin to the stem by diffusion through a transverse basal surface only. To achieve this the sides of the basal part of the stems of cuttings were coated with cocoa butter. The permeability of cocoa butter to auxin was first tested in the following manner. Three groups of single-node cuttings, with 2 cm. of stem above the bud and 4 cm. below were treated thus.

The portion of the stem above the leaf was dipped for 1.5 cm. of its length into just solidifying cocoa butter. In two of the groups the cocoa butter was not touched further; in the third group, just before

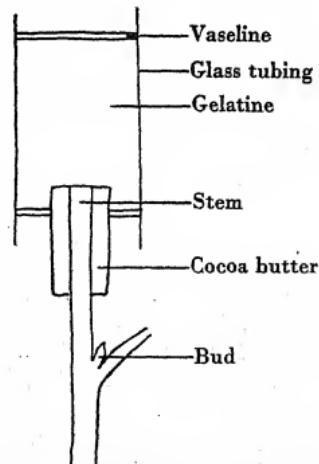


Fig. 2

the butter had completely solidified, a slice of the stem with the covering cocoa butter was removed by a transverse cut across the end of the stem. One of the groups with untouched cocoa butter was left as a control, in the other untouched group and in the third group the stem top was further covered with gelatine containing strong hetero-auxin in small glass tubes (Fig. 2). The gelatine in the tubes was just warm and liquid at the time of application, and when it solidified it was covered with vaseline to prevent drying out. The gelatine never reached farther down the stem than the cocoa butter. The length of the buds were as follows in the three groups in three separate experiments. In the first experiment the concentration of hetero-auxin was $1 \text{ in } 10^4$, and in the other two $4 \text{ in } 10^5$.

TABLE VIII. *Length of buds in mm. at end of experiment*

Duration of experiment	Control	Hetero-auxin applied to end covered with cocoa butter	Hetero-auxin applied to exposed apical cut surface
8 days in November	6.5, 1.5, 8, 7	4.5, 5.5, 5.5, 4	1.5, 1, 1, 0.75
6 days in May	5, 6.5, 6, 7, 6.5	5, 4.45, 4.5, 6	2.5, 2.5, 2, 2.5, 2
6 days in May	7.5, 3.5, 8, 9, 10, 4	7.5, 8.5, 7, 3.5, 6, 5	2, 2, 1, 1.5, 2.5, 1

The difference between the controls and the group coated with cocoa butter does not seem to be sufficient to suggest any appreciable leakage of hetero-auxin through the cocoa butter.

The main experiment was then set up with two kinds of cuttings, upright and inverse. These two groups differed in the length of stem above the bud, as well as in their position in the specimen tubes. The "upright" cuttings had 4 cm. of stem below the bud and 2 cm. of stem above the bud, and stood with their bases in water. The "inverse" cuttings had 2 cm. of stem morphologically below the bud, and 4 cm. above, and stood with their apical ends in water. The cuttings were arranged in three matched groups, one inverse and two upright. One inverse group was left as a control (being placed inverse to give a minimum bud growth for the control), and the two other groups were treated with cocoa butter and auxin gelatine on their physically upper ends. A slice of stem near the upper end was removed by a cut through the butter, just as in the third group of the last experiment (Fig. 2).

Table IX gives the average results in six experiments, each comprising two groups of experimental plants and one group of controls. There were six or seven plants in each group. The cuttings were all made from the node belonging to the second youngest fully expanded leaf. The initial length of the buds was about 1 mm.

TABLE IX. *Average lengths of buds in mm. at end of experiment*

Duration of experiment in days	Hetero-auxin strength	Control	Inverted with hetero-auxin in gelatine	Upright with hetero-auxin in gelatine
10	2 in 10^5	8.2	2.7	1.7
10	2 in 10^6	7.5	4.6	2.7
8	2 in 10^5	9.6	5.2	2.9
10	1 in 10^5	7.2	4.4	2.9
7	1 in 10^6	6.0	3.9	1.7

In all these experiments inhibition varies between the three groups in a constant order. In experiments where the hetero-auxin is applied to the morphologically upper surface the buds elongate by about 2 mm. only. Inhibition is almost complete. A certain amount of inhibition is also apparent when hetero-auxin is supplied to a morphologically basal cut surface; for in these experiments the buds elongate by about 4 mm. only. This bud length is considerably less than that of the control buds which had reached lengths of about 8 mm. at the end of the experiment. The inhibition of the buds of the inverted cuttings was contrary to expectation, but the values may even be too low, as in this group of experiments some small bubbles of gas were exuded from the centre of the stem into the gelatine. Thus the auxin in this case must have passed at least a small way up the stem against the polar mechanism. These results, which are not complicated by the transpiration stream, still do not completely exclude the direct theory of inhibition by auxin. For they show that some hetero-auxin must be able to enter the cuttings by diffusion through the morphologically basal cut surface, even against the normal direction of transport, although it then inhibits the axillary bud less strongly than when it enters by the apical cut surface. Accordingly the following experiments were undertaken in order to estimate how much auxin enters an inhibited shoot.

(ii) Auxin in inhibited shoots

The question of the amount of auxin in inhibited shoots was thought to be relevant to the question of auxin action. If there is an appreciable amount present or if the polarity of the inhibited shoot is reversed the direct theory will receive support. Inhibited and normal internodes were tested for auxin in the following manner. A two-shoot pea plant (Snow, 1931) was produced by removing the young main shoot of a seedling and allowing the cotyledonary buds to develop. In many cases one of the two shoots partially inhibited the other. In these plants 5 mm. of the youngest internodes from

both the shoots were tested for auxin on oat coleoptiles. (The longer shoot was tested when it had four or five fully expanded leaves, the shorter shoot had then expanded two or three leaves only.) The oat coleoptiles grown in the dark were decapitated, the included leaf was pulled out from the base, and the length of pea stem fixed eccentrically on to the cut surface of the coleoptile with a little dilute gelatine. The experiments were carried out at a temperature of 68°C. The following figures (Table X) show the curvatures of the coleoptiles 3 hours after the stems had been applied to them. The curves were all negative except where otherwise indicated.

TABLE X. Curvatures of coleoptiles after 3 hours

	Normal shoot applied	Inhibited shoot applied	
		Inverted	Upright
Exp. a	20°, 45°, 30°, 40°, 15°	0°, 0°, 0°, 0°, 0°	0°, 0°, 0°, 0°, 5°
Exp. b	20°, 35°, 20°, 30°, 10°	0°, 0°, +5°, 0°, 0°	0°, 0°, 0°, 0°, +2.5°

The auxin content of older internodes was tested by taking 5 mm. length of the lowest internode from both shoots of two-shoot plants. The longer shoot had expanded four leaves and the shorter shoot two. The following are the coleoptile curvatures after 3 hours (Table XI).

TABLE XI. Curvatures of coleoptiles after 3 hours

	Normal shoot applied	Inhibited shoot applied	
		Inverted	Upright
	10°, 5°, 10°, 10°, 0°	0°, 0°, 2.5°, 0°, 0°	0°, 0°, 0°, 0°, 2.5°

Experimentally inhibited shoots were also tested. They were produced by decapitating one shoot of a two-shoot pea plant whose shoots were exactly equal (compare Snow, 1931). The plants were then left in the greenhouse for 7 days and similar internodes tested on oat coleoptiles. The buds on the decapitated shoot had not grown out, indicating that the shoot was being inhibited by its twin. Table XII shows the bend of the coleoptile 3 hours after the beginning of the experiment. Internode 4 was used from both shoots. Both shoots had expanded four leaves before the one shoot was decapitated.

TABLE XII. Curvature of coleoptile after 3 hours

	Normal shoot applied	Inhibited shoot applied	
		Inverted	Upright
	15°, 5°, 15°, 0°, 0°	0°, 0°, 0°, 0°, 0°	0°, 0°, +2.5°, 0°, 0°

The results of the above three tables show that the youngest internode of a growing shoot contains a considerable amount of auxin (since the negative curves are due to acceleration of growth on the side to which the piece of internode was applied), whereas a similar internode of its inhibited twin contains none. Older internodes of a twin-shoot plant show considerable but less auxin in the normal growing shoot, and again none, or scarcely any, in the inhibited shoot.

The absence of auxin from an inhibited shoot certainly suggests that inhibition is a secondary or indirect process.

Some experiments were also set up to determine in what quantities auxin is transported by normal and by inhibited stems. These were performed by placing a small portion of hetero-auxin paste of concentration 1 in 10^4 , on one end of a piece of pea stem 4 or 5 mm. long. The piece of stem was then placed eccentrically on oat coleoptiles, as in the previous experiments. In numerous experiments with pieces taken from normal growing shoots, it was found without exception, in agreement with Thimann and Skoog (1934 a) and others, that pieces taken from normal growing shoots transported the hetero-auxin actively, but only in the morphologically downward direction. The negative curvatures caused in the coleoptiles, when transport through the pieces of stem was in the downward direction, were usually about 40° and seldom less than 30° .

The following experiment, which needs to be repeated, indicates that an inhibited shoot transports hetero-auxin much less actively than a growing one. In this experiment lengths of 5 mm. of internode 1 were used, and the strength of the hetero-auxin paste applied was again 1 in 10^4 . The following (Table XIII) are the negative curves of the coleoptiles after 3 hours.

TABLE XIII. *Transport through inhibited shoot*

Upright $0^\circ, 0^\circ, 5^\circ, 5^\circ$	Inverted $10^\circ, 0^\circ, 5^\circ, 0^\circ$
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The transport experiment also suggested that the polarity of a stem cannot be reversed by the application of strong hetero-auxin paste (1 in 10^4) to its basal end. This point is worth noting, since the supporters of the "direct" theory of inhibition might seek to explain by means of an induced reversal of polarity the results of one of the groups of experiments in this paper—namely those in which rather strong solutions of hetero-auxin in gelatine were applied to a cut surface which was morphologically basal.

The fact that transport experiments, such as those just described, all show that normal growing stems transport auxin only in the morphologically downward direction is another difficulty for the "direct" theory of inhibition by auxin—especially when it is remembered that not only lateral buds but lateral shoots can be inhibited. But it is perhaps a less serious difficulty than the fact that inhibited shoots, as shown above, contain hardly any auxin. For the results already reported in Table VIII show that in some circumstances some hetero-auxin must be transported in the upward direction for a short distance at least, since it can enter a stem cutting by diffusion from gelatine through the basal cut surface, and bring about partial inhibition of the axillary bud above.

DISCUSSION AND CONCLUSION

The chief point evident from the above experiments is the ability of hetero-auxin to inhibit the growth of young internodes of *Pisum sativum*, if it reaches them from a position morphologically below them. The results agree with Sniow's experiments (1931) with twin-shoot plants which showed that an influence produced by one growing shoot could inhibit the growth of a side shoot as well as its own axillary buds. This inhibition of stem growth by auxin shows that the inhibition is not due to some response peculiar to buds alone.

The minimum amount of auxin necessary for complete stem inhibition is greater than that necessary for bud inhibition. This is not strange in view of the relative vitality of the two organs; for it is to be supposed that a large growing organ such as the young internode will have more resistance to an external antagonistic influence than the small already dormant bud: indeed it is perhaps surprising that the minimum strength for bud inhibition is only about ten times less than for stem inhibition, being $5 \text{ in } 10^6$ for stem inhibition (Exp. 3) and $4 \text{ in } 10^6$ for bud inhibition (Exp. 1).

The nature of auxin action, whether it is to be acceleration or inhibition, appears to be determined by one thing—the position of the auxin source relative to the organ to be affected. At the present time acceleration of stem growth has been demonstrated only when auxin is travelling morphologically downwards, and inhibition of growth when the auxin is coming from a position morphologically basal to the inhibited part. The two different effects do not appear to be connected with any variations in auxin concentration, for the same concentration of hetero-auxin ($2 \text{ in } 10^5$ in lanoline) can either

inhibit or accelerate growth, according as it is applied from below or above. This being so, there are two possibilities as to the actual nature of inhibition by auxin: either the auxin itself travels upwards and has a different effect from the same substance travelling downwards, or the auxin itself travelling only downwards produces indirectly a secondary inhibiting effect which can travel in either direction. Against the first alternative (the direct theory) are the two objections already discussed in the introduction with the added difficulty of supposing that a shoot of a twin-shoot plant as well as a lateral bud is unable to use up auxin from the other shoot. Another form of the "direct" theory, the possibility of auxin having two forms, one of which travels upwards inhibiting and the other downwards accelerating growth, is difficult to disprove; but the absence of any auxin in an inhibited shoot militates against it as also against Thimann and Skoog's form of the "direct" theory.

The question of auxin transport and its bearing on the problem is discussed with the experimental work: the conclusion reached is that the mainly polar (downward) transport of auxin in the stem is against the "direct" theory.

The indirect theory remains, but there is not yet any evidence of any special substance capable of inhibiting, and produced secondarily by auxin action. The experiments here reported show that there is very little auxin, if any, in inhibited shoots, but it is difficult to show that there is absolutely none. But so far as they go these experiments are strongly in favour of the "indirect" theory.

Thimann and Skoog object that Laibach's primary growth reaction is not apparent in the pea plant, as they find (1934 *a, b*) that even a decapitated stem contains enough auxin to elongate to its maximum. But it does not seem necessary to postulate externally visible growth, since even if the primary reaction is to be one of growth the cambial divisions caused by auxin, as shown by Snow (1935) and also found by the writer in pea plants, would fit the situation.

Stems without leaves are more susceptible to inhibition by auxin influence than those with leaves, and this may possibly be due to protection by the auxin produced by the leaves themselves.

SUMMARY

1. Axillary buds of *Pisum sativum* present on single-node stem cuttings are inhibited if the cuttings are placed with their ends in hetero-auxin solution.

2. The growth of the young stem is inhibited if whole shoots are placed with their bases in hetero-auxin solution.
3. The growth of the young internodes is inhibited by lanoline containing hetero-auxin placed on the stem in a position morphologically below them, although their growth is accelerated by the same hetero-auxin paste when applied to them from above.
4. Leaves protect a stem against inhibition by hetero-auxin.
5. Buds of cuttings are inhibited by hetero-auxin in gelatine applied to the cut ends of the stems above or below them, but more strongly if the hetero-auxin is applied from above.
6. There is little or no auxin present in an inhibited shoot.
7. A completely inhibited shoot transports auxin only very feebly in either direction, if at all.
8. It is concluded that inhibition of lateral buds and shoots is probably a secondary process, which originates from some positive primary process promoted by auxin in the inhibiting shoot.

Grateful thanks are owing to Dr Weissberger of the Dyson Perrins Laboratory, Oxford, who kindly prepared the synthetic hetero-auxin, and to Mr R. Snow who suggested and supervised the work.

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MECHANICAL FACTORS IN THE DISTRIBUTION OF A BLUE-GREEN ALGA,
RIVULARIA HAEMATITES

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(With 4 figures in the text)

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INTRODUCTION

DURING a visit to a limestone spring between Ohrid and Resan in southern Jugoslavia, it was observed that the gelatinous masses of *Rivularia haematites* were not distributed uniformly over the length and breadth of the stream, but were confined to certain areas. Closer examination showed that within the areas in which the alga occurred, the size of the thallus was in some cases related to the size of the stone to which it was attached, but in other cases this was not so. In the following account an analysis is made of the factors involved in the distribution of the plant, and an explanation of the correlation or non-correlation between stone size and thallus size is suggested. The results seem to provide a clear example of the dependence of this distribution on relatively simple mechanical factors—a phenomenon for which quantitative evidence is rare in ecological literature.

THE ENVIRONMENT

The bottom of the stream varied from mud, through coarse sand, to gravel and small stones (*ca.* 5 sq. cm. in area), large stones (*ca.* 40 sq. cm. in area) and flags of limestone. The vegetation showed a

zonation from *Apium*, in coarse sand and submerged gravel, to *Juncus*, *Mentha* and *Carex* spp. on raised gravel beds. On exposed stones in the fast-flowing parts of the stream were clumps of *Vaucleria* and moss. In one sheltered, shallow, and mud-bottomed

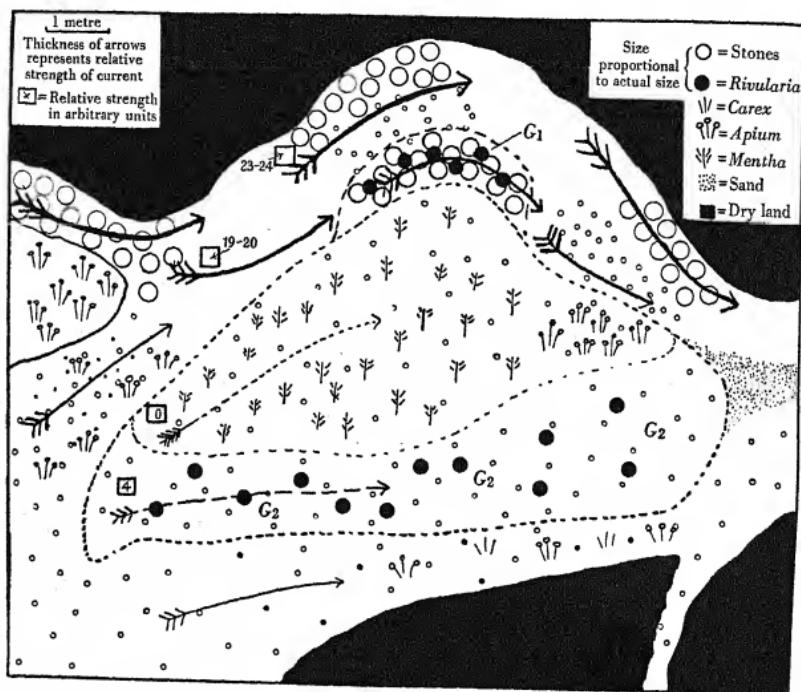


Fig. 1. Sketch map of part of a stream between Ohrid and Resan (Yugoslavia) showing the correlation between the size of stone, velocity of stream and area of thallus in the distribution of *Rivularia haematites*. The numbers are measurements of velocity expressed in arbitrary units. G_1 and G_2 mark the areas from which the data for Figs. 3 and 4 were taken.

stretch, masses of *Draparnaldia* clung round the clumps of *Juncus*. *Rivularia haematites* occurred only on small stones, large stones, and flags of limestone, never on sand or gravel. In Fig. 1 is shown a sketch map of one stretch of stream, selected on account of the great range of conditions shown in a small area. The limited distribution of *Rivularia* is plainly seen from this map.

SUGGESTED FACTORS IN THE DISTRIBUTION

The distribution of the alga may be explained in terms of the following factors:

- (a) The growth form of the thallus.
- (b) The tensile strength of the attachment.
- (c) The velocity of the stream.
- (d) The size of the stone to which the thallus is attached.
- (e) The size of the thallus.

(1) The growth form of the thallus and the strength of the attachment

If the clumps of alga are examined it is found that the area of attachment of the thallus to the substrate is not the same as the area of the clump, but is much smaller, since the plant grows as a bubble

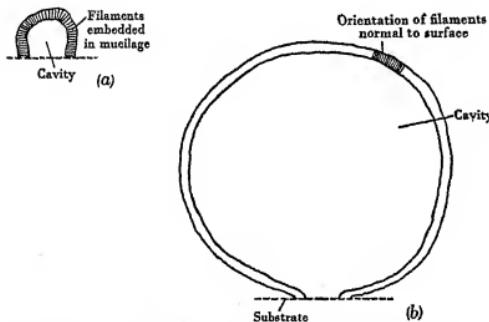


Fig. 2. Diagrammatic representation of the growth of the thallus in *Rivularia haematis*. Although the bulk of the thallus has increased considerably between (a) and (b), the area of attachment is unchanged. The thallus grows in the plane at right angles to the axis of the filaments.

which collapses under its own weight. This bubble structure of the thallus is seen clearly in small, and presumably young, growths which have the form of hollow hemispheres attached to the substrate by the rim, and having the filaments orientated at right angles to the surface of the thallus (see Fig. 2). Such a structure may result from the fact that the filaments of *Rivularia* do not grow indefinitely in a direction parallel to the axis of the filament; the length of a filament is limited. Accordingly the rate of growth of the thallus in the plane at right angles to the axis of the filaments, is greater than the rate of growth in the plane parallel to the axis of the filaments. Growth in

the former plane depends on frequent false branching and the swelling of the interstitial mucilage. Now since the filaments tend to lie flat on any surface with which they are in contact, the attachment of the thallus will consist of filaments lying side by side with their axes parallel. Therefore with reference to the substratum, the thallus will tend to increase in area in the plane at right angles to the substrate and to the axis of the filaments, rather than in the plane of the substrate. In other words, a circular thallus will not grow outwards in continuous attachment (like a lichen thallus) but will grow as a bubble. All that has been said so far can be confirmed by examining the structure of macroscopic thalli.

The important conclusion from this examination of the growth form of *Rivularia* is that the surface area of the thallus increases at a greater rate than the area of attachment (this is shown in Fig. 2). Therefore, if the clump continues to grow in a stream of water of constant velocity, in all probability a time will come in the history of the thallus when the force (proportional to the size of the thallus) tending to detach the plant from the substrate, will exceed the tensile strength of the attachment. There should therefore be a difference between the maximum size of thalli from parts of the stream with different velocities. In Table I are shown the approximate areas of clumps from very fast, fast and slow stretches of the stream. It is

TABLE I
Area of thallus in sq. cm.

Very fast = 24	Fast = 20	Slow = 4
6	2	5
6	2	8
16	6	10
36	7	10
	10	10
	26	12
	28	18
	30	20
	35	23
	90	30
		90

Velocities expressed in arbitrary units.

apparent that the maximum size of the thallus is least in the fastest portion of the stream. (The areas were calculated by treating the thallus as a rectangle and determining the mean length and breadth.) The small number of measurements in the first column is due to the fact that there were only four clumps of alga to be found in the fastest stretch, whereas there was no difficulty in finding ten clumps in a given stretch at slower speeds. We have then as a consequence of

the growth form of the thallus a correlation between stream strength and the frequency of occurrence and maximum size of the thallus. In the case of a true encrusting form in which the area of attachment increased with the area of the thallus, and in which the thallus was in continuous contact with the substrate, the size of the thallus would not be limited by the current strength.

(2) *The relation between the size of stone to which the thallus is attached and the velocity of the stream*

In Table II are recorded the areas of stones on which the alga was growing in very fast, fast and slow portions of the stream. The observed correlation of stone size with current strength is not an

TABLE II

Area of stone in sq. cm.

Very fast = 24	Fast = 20	Slow = 4
50	3	1
80	8	2
150	21	3
480	35	4
	36	5
	41	5
	48	7
	50	9
	50	9
	63	10

expression of the fact that only large stones are found in the fast regions (and therefore only large stones are available for the alga) since, as can be seen from Fig. I, small stones also occur in fast stretches, and from these the alga is absent. (The size of the stones was determined from their approximate area treating them as rectangles. They were mostly derived from slabs of stone of approximately the same thickness.) This correlation of current strength and stone size may be due to the fact that as the thallus grows its lifting power increases, until a thallus on a small stone may cause the stone to be swept away down stream to a place in which the thrust of the current on the thallus is no longer sufficient to lift the stone.

(3) *The relation between the size of the thallus and the size of the stone to which it is attached*

If the interpretation of the correlation of stone size and current strength is correct, there should be some relation between the size of the thallus and the size of the stone on which it is growing (in a

stream of given velocity). Fig. 3 shows this relation for plants growing in the region G_1 of Fig. 1. The area of the thallus is obviously proportional to the size of the stone to which it is attached. It might be argued that this was because a larger stone permitted a greater area of attachment, but it has been pointed out that the area of attachment is not directly proportional to the size of the thallus. The fact that large thalli can grow on small stones is established by measurements made in the region G_2 . In Fig. 4 the relation between thallus size and stone size is shown for the region G_2 —a region in which the current strength was much less than in G_1 . Here it is seen that not only can large thalli grow on small stones, but that when the current strength decreases, the proportionality between thallus size and stone size disappears. At the other end of the current strength range, i.e. in very fast regions, the number of measurements is too small to justify any conclusion on the relation between stone size and thallus size.

To complete the analysis it remains to add that as a corollary of the fact that stream velocity limits the maximum size of the thallus developed in a given region, thalli which exceed this limit will be torn from their attachment when the stone on which they grow is so large that the breaking limit of the attachment is reached before the thrust on the thallus is able to move the stone. In the region marked G_2 many unattached thalli were found. These had presumably been torn off in faster reaches, and deposited by the stream when the velocity fell.

The experiment was made of transferring small stones, with large thalli attached, to more rapid stretches of the stream. They were immediately swept down stream and deposited in slower regions. The absence of the alga from sand and gravel stretches is quite understandable when it is realised that these were usually found in fairly swift regions of the stream. When the gravel patch lay in a slower stretch (as in G_2) the alga was able to grow.

SUMMARY

The distribution of *Rivularia haematis* may be explained in terms of mechanical factors.

(1) The growth form of the alga is interpreted as the consequence of the properties of the filaments.

(2) An attempt is made to explain the fact that the bulk of the alga increases more rapidly than the area of attachment.

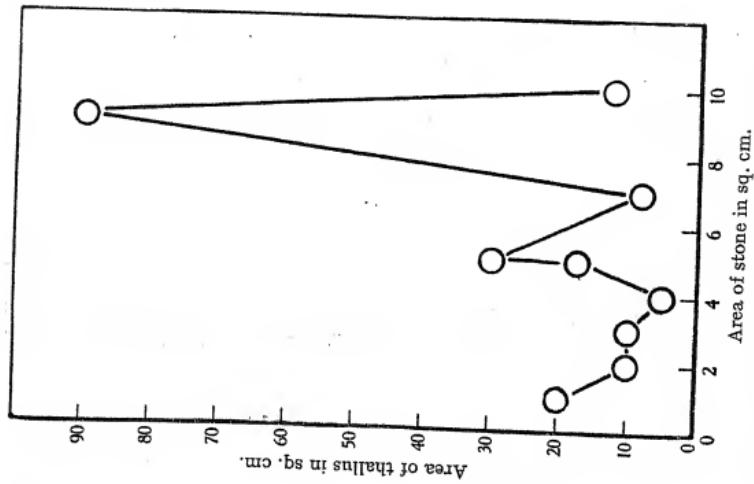


Fig. 3. Relation between area of thallus and area of stone to which it is attached in fast stretch (region G_1 of Fig. 1). Area of thallus increases with stone size.

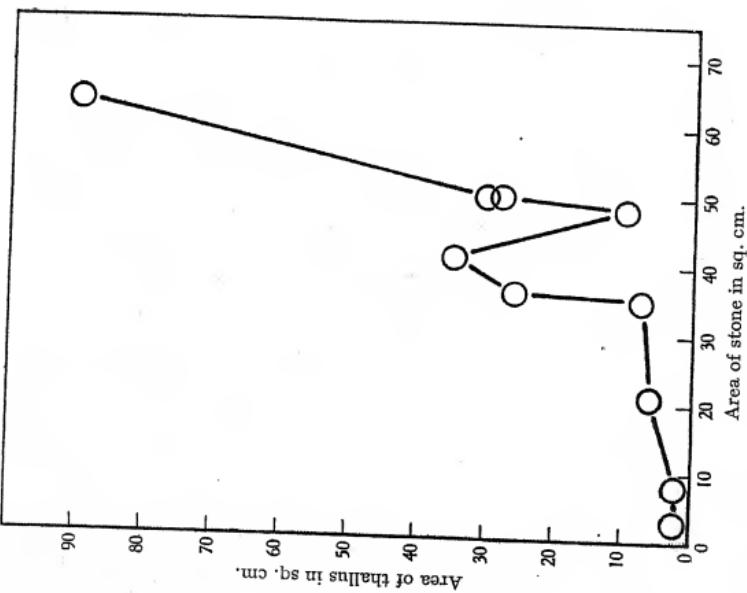


Fig. 4. Relation between area of thallus and area of stone in slow stretch (region G_1 of Fig. 1). No correlation between thallus size and stone size.

(3) The velocity of the stream limits the maximum size of thallus in a particular region. Thalli exceeding this limit are either (a) torn from their attachment (if the stone is very large), or (b) carry their stone with them to a slower part of the stream, if the thrust on the thallus is able to shift the stone (i.e. if the weight of the stone is less than the breaking stress of the attachment).

(4) It is shown that in a given region of relatively fast flow, the thallus size is proportional to stone size.

(5) When the flow is much slower, thallus size is independent of stone size.

(6) It has been shown experimentally that small stones with thalli attached are only in stable mechanical equilibrium in their particular environment. When transferred to faster stretches they are swept away.

I am indebted to Mr M. G. M. Pryor for the measurements of current velocities, and to Mr P. Ulljott for permission to check the sketch map in Fig. 1 against his more accurate map of that region.

SEASONAL CHANGES IN THE CARBOHYDRATES OF THE WHEAT PLANT

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(With 9 figures in the text)

INTRODUCTION

THE data accumulated during two years' observations on the carbohydrate contents of wheat plants growing under field conditions render it possible to present a survey of the drifts of the various carbohydrates within the plants and the problems that arise from their study. The work is preliminary to a projected comprehensive study of the metabolic development of a crop plant throughout its life cycle and represents an approach to the problem of describing in quantitative chemical terms the sequence of events occurring during the growth, maturation and reproduction of a plant and also the reactions of varying external factors on this sequence.

Wheat was selected as the crop plant for study in view both of its economic importance and the considerable amount of knowledge concerning its morphological development already in existence. It is proposed to follow the study of the drifts of various carbohydrates in the whole plant with further studies dealing with the distribution of carbohydrates within the various parts of the plant at different stages of the plant's development, and also with studies in the daily variation of the carbohydrate content of the plant and its parts.

Materials, sampling and estimation methods

Two varieties of wheat were selected: (a) Rivets, a *turgidum* wheat whose cultivation is restricted to the heaviest clay soils, and (b) Wilhelmina, a *vulgare* wheat which is usually grown on less heavy soils. The two wheats, although both capable of high yields, differ considerably in appearance. In the season 1932-3 these two wheats were grown¹ on separate fields (Rivets on Stickfast and Wilhelmina on Gault-Pit on the Cambridge University Farm).

¹ Rivets drilled October 5, 1932, seed-rate: 3 bushels per acre. Wilhelmina drilled October 28 and 29, seed-rate: $2\frac{1}{2}$ bushels per acre. Both followed on normal field cultivation.

For sampling each field was divided into five strips of approximately equal area. Then samples were taken from each field; each consisting of ten 1-ft. row units taken at random, two from each strip. The ten units in each set were grouped together to give a single sample from a field at fortnightly or weekly intervals throughout the season.

For the season 1933-4 two acres of land (on Girton Allotment) were divided into four equal strips and the Wilhelmina and Rivets grown¹ on alternate strips. Each strip was subdivided into five plots of equal area and a 1-ft. row unit taken at random from each plot, thus samples of ten 1-ft. row units were again obtained for each variety. These units were grouped together and two such grouped samples obtained for each variety at each time of collecting.

Sunrise was selected as the most suitable time of the day for sampling, the major consideration being that of securing the maximum possible degree of uniformity between successive samples in respect of external conditions to determine the presence of any developmental drift of the carbohydrates. It was decided that a few hours of darkness before each sampling time would give more nearly constant conditions of "lighting" and temperature than several hours of light having different intensities and different qualities before each sampling time.

After collection each sample was weighed, the roots removed and the plants cut into small pieces with scissors or shears and placed in hot spirit (diluted to give, with the water in the plant material, an approximate concentration of 80 per cent.). With the large samples obtained towards the end of a season a subsample of the well-mixed plant material was weighed out and placed in hot spirit. When ears appeared on the plants they were separated from the plants and dealt with as distinct samples. Determinations of the tissue water content were obtained from subsamples retained from each sample. The Kilner jars in which the samples were placed were carried to the laboratory as quickly as possible, and the samples boiled on a water-bath under reflux condensers. At this stage the samples were stored in a refrigerator to await analysis.

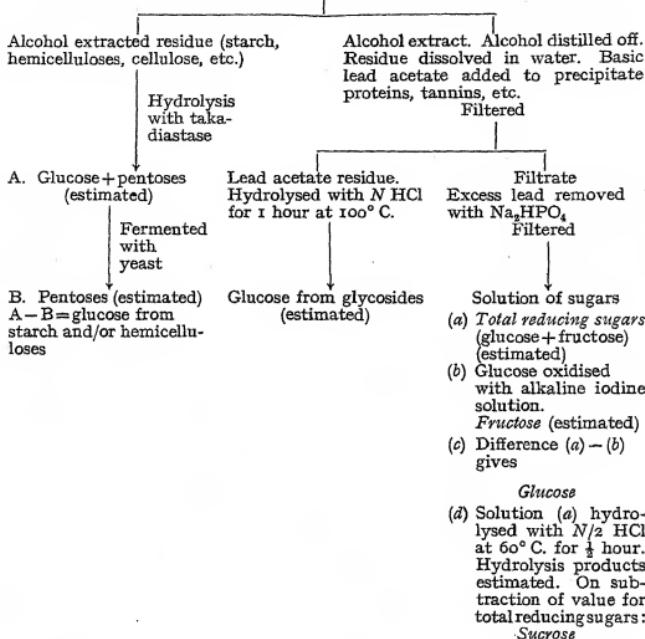
The methods used in the preparation and estimation of the various carbohydrates in the plant material are shown in outline in the scheme shown on p. 231.

All the carbohydrates were estimated in the form of reducing

¹ Both drilled on October 13, 1933. Seed-rate: 2 bushels per acre. Followed experimental bean plots.

Seasonal Changes in Carbohydrates of Wheat Plant 231

The enzymes of the fresh plant tissue were deactivated by placing tissue in boiling alcohol. Sugars, etc., were extracted with 80 per cent. alcohol



sugars by the Shaffer-Hartmann method as modified by Maskell (not yet published) and the data expressed in terms of glucose.

With the exception of ears from 3 to 4 weeks after their emergence onwards no unquestionable positive test was obtained with iodine for the presence of starch in the alcohol-extracted residue of the plant tissues. However, starch determinations were carried out on all samples using a taka-diastase hydrolysis method (0.5 gm. dried alcohol-extracted residue boiled with 10 ml. water for 1 hour and then incubated at $35^\circ C$. for 48 hours with 5 ml. of 1 per cent. filtered undiluted taka-diastase,¹ 4 c.c. of acetate buffer $pH=4.7$ and 1 ml. of toluene), which with moderate amounts of starch gives complete hydrolysis to glucose (Maskell and El Gawadi, Denny). The reducing substances in the hydrolysis products were estimated using a blank

¹ The taka-diastase used for the hydrolysis was a special undiluted preparation obtained from Messrs Parke, Davis and Co.

containing taka-diastase but not plant tissue, since a small amount of reducing substances is present in the taka-diastase. The hydrolysis products were fermented with a commercial brand of bakers' yeast which could ferment glucose, fructose and sucrose completely but did not affect pentoses during the time period used.¹ The fermented liquor gave a strong positive test with Bial's Reagent for the presence of pentoses (arabinose or xylose), so the estimate of the amount of reducing substances present after fermentation furnishes an indication of the amount of pentoses liberated from hemicelluloses and pentosans on taka-diastase hydrolysis. All the reducing substances in the taka-diastase hydrolysis products were completely oxidised by the standard alkaline iodine method for glucose oxidation. This indicates the aldo constitution of both the fermentable and non-fermentable fractions of the hydrolysis products, agreeing with the conclusion that the non-fermentable reducing substances are pentose sugars and that the fermentable reducing substance is glucose derived from hemicelluloses or starch (when present). The various fractions obtained from the alcohol extracted residue will be described as "taka-diastase hydrolysis products", "fermentable taka-diastase hydrolysis products", and "non-fermentable taka-diastase hydrolysis products" in view of the uncertainty as to the origins of the sugars estimated under these titles.²

Weather

Both the seasons 1932-3 and 1933-4 were relatively dry. During but 3 weeks in 1932-3 and but one in 1933-4 did the weekly rainfall in Cambridge exceed 1 in. Periods of low temperature occurred in both seasons during the months of December and January—the frost in late January 1933 being prolonged and associated with a fairly high record of hours of sun for the time of year. In a later section attempts are made to correlate the variations in cane-sugar

¹ To 20 ml. of the hydrolysis products 10 ml. of a 10 per cent. suspension of washed yeast were added together with 2 ml. of acetate buffer $pH=4.7$ and 2 ml. of 0.1 M KH_2PO_4 solution. After 3 hours' incubation at 35° C. the yeast was coagulated with alumina cream and filtered off. The non-fermentable reducing substances were estimated by the modified Shaffer-Hartmann method. The blank consisted of the taka-diastase blank which had been submitted to the full fermentation procedure.

² No systematic search was made for the "levulosanes" or fructosans (fructose-yielding polysaccharides) described by Belval (1924) as occurring in wheat. The application of the methods used by Cugnac (1931) showed no trace of their presence in the alcohol extract but their presence in the extracted residue has been demonstrated. Estimation of the amounts of these fructose-yielding polysaccharides in various parts of the wheat plant is in progress.

content with weather conditions to determine the extent of the effect of environmental conditions on the underlying drift of this sugar in the plant.

DRIFT OF WATER CONTENT, TOTAL ALCOHOL-SOLUBLE SUBSTANCES,
SUGARS, POLYSACCHARIDES AND GLYCOSIDES THROUGHOUT THE
SEASON

Water content

The drifts of water content of both varieties, Rivets and Wilhelmina, throughout the two seasons are shown in Fig. 1. The curves for the 1933-4 season fluctuate less than those for the previous season, due mainly to the use of larger samples for the dry-weight determinations.

During winter and early spring the water content of both varieties varied between 80 and 85 per cent., and in 1933-4 that of Wilhelmina was slightly but consistently lower than that of Rivets. There were abnormally low values for both varieties at the April 25, 1934, sampling date, which are apparently related to an unusually high relative growth-rate of the plants at this time.

Values for the relative growth-rate for several weeks preceding this date and for several weeks following it are given in Table I. The rate for the fortnight from April 11 to April 25 is seen to be approximately four times as great as in the periods immediately preceding or following this interval.

TABLE I

Relative growth-rate.¹ Dry weight increase per gm. per week

Period	Rivets	Wilhelmina
Feb. 21-Mar. 7	0·05	0·08
Mar. 7-Mar. 22	0·10	0·11
Mar. 22-Apr. 11	0·21	0·17
Apr. 11-Apr. 25	0·83	0·84
Apr. 25-May 9	0·15	0·20
May 9-May 23	0·04	0·06
May 23-June 6	0·29	0·25

¹ Data for relative growth-rates were obtained from the values of the dry weight of plant material per 10-ft. row given in Tables II and III respectively.

From the end of April in both seasons the water contents of both varieties fell uniformly, presumably indicating considerable differentiation of parenchymatous tissues into fibres, conducting elements, etc. The vertical lines drawn through the curves on June 7 in 1933 and June 20, 1934, mark the dates on which the ears were first

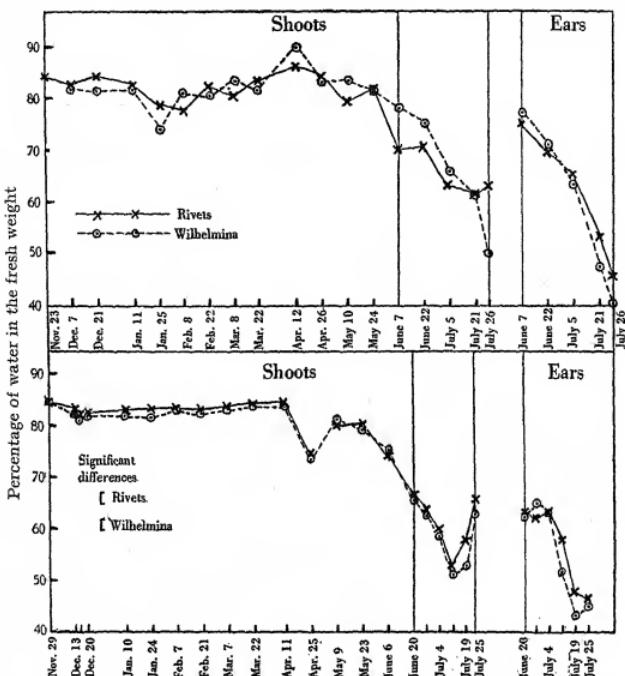


Fig. 1. Water contents of Rivets and Wilhelmina wheats. Top curves for 1932-3 season, lower curves for the 1933-4 season. Curves for the ears and shoots with ears removed are shown separately after June 7, 1933 and June 20, 1934. The magnitudes of differences which correspond to a $P=0.05$ level of significance¹ are shown for the 1933-4 curves as vertical lines.

¹ These values for the significant differences ($P=0.05$) are obtained as follows. Each "observation" as represented by a point on the curve is the mean of the determinations of two independent samples. Hence:

$$\text{Variance of a single "observation"}, V_a = \frac{SS (x - \bar{x})^2}{2n},$$

where $(x - \bar{x})$ = deviation of any determination from the corresponding duplicate mean, and n = number of "observations" (duplicate means).

Variance of difference between any two "observations" = $2V_a$.

Significant difference ($P=0.05$) = $t \times \sqrt{2V_a}$,
where t is the value for $P=0.05$ and n degrees of freedom given in Fisher's (1930) table of t .

removed from the samples and treated separately. The curves following these dates are for the shoots without ears, the curves for ears alone being given separately.

In the 1933-4 season the water contents of Rivets and Wilhelmina shoots fell to 52.9 and 51.1 per cent. respectively on July 11, and then the water contents of both varieties rose reaching 66.3 and 63.4 per cent. respectively on July 25. This increase must be ascribed to rain after drought; there was no rain between July 1 and 11, and then between July 11 and the next sampling date, July 19, 0.38 in. fell, and between July 19 and 25 0.95 in.

In the ears the water contents were approximately the same as in the shoots at the first time of separation in each season (see separate curves for ears in Fig. 1), and fell on succeeding sampling dates reaching final values of 45.8 per cent. for Rivets and 40.4 per cent. for Wilhelmina in 1933. In 1934 there was little change in the water content between June 20 and July 4, but then it decreased, that of Wilhelmina remaining consistently lower than that of Rivets. Wilhelmina showed a slight rise in water content and Rivets a decreased rate of drying out between July 19 and 25, this effect on the ears, of the rain following drought, being less marked than on the shoots.

It is clear that there is a definite seasonal trend of water content in the wheat plant consisting of a decreasing amount of water relative to total plant material during the phases of vigorous plant growth and development of the ears. Data for carbohydrates, etc., expressed as percentages of the plant fresh weight will therefore be based on a standard which itself has a definite seasonal drift and therefore must be taken into account before interpretation of the observed drifts is attempted. Expression of data as percentages of plant dry matter, residual dry matter, or of the water content would remove the arithmetical effect of this underlying drift but are, perhaps, not so generally satisfactory as the presentation of values for the amounts of particular substances relative to the total amount of fresh plant material, and this latter method will be adopted in the present paper.

Total alcohol-soluble substances (sugars and non-sugars)

Records of the total alcohol-soluble substances in the two varieties of wheat were kept for the 1933-4 season only. The drifts for the total substances and also for the component total sugar (sucrose, glucose and fructose) and non-sugar fractions are shown in

Fig. 2. The non-sugar fraction contained glycosides, amino acids, some proteins (prolamins), etc. The ears were separated from the shoots on June 20, and the curves then continue for the shoots alone, values for the ears alone are shown separately in the figure.

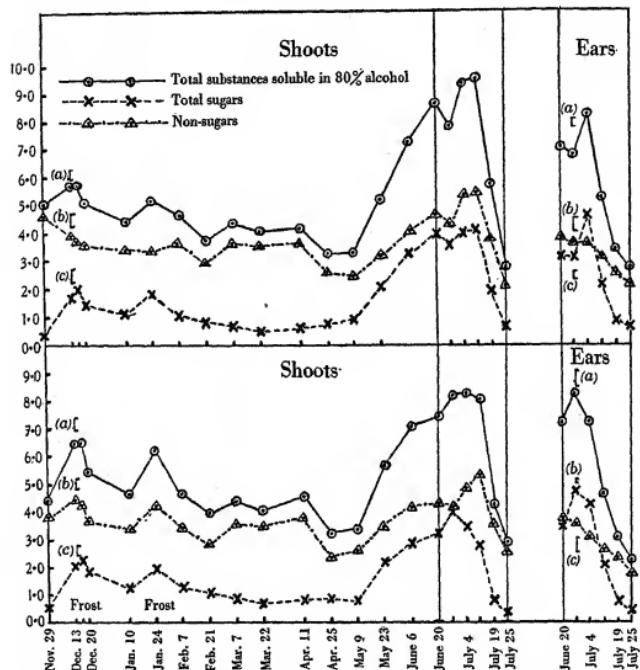


Fig. 2. Drifts of total alcohol-soluble substances and the component fractions, total sugars and non-sugars, during 1933-4. Top curves are from Rivets and the lower from Wilhelmina. Values for the ears and the shoots with ears removed are shown separately after June 20, 1934. The vertical lines labelled (a), (b), (c), for each set of curves give the minimum significant difference, (a) of total alcohol substances, (b) of total sugars, and (c) of non-sugars.

The shoot. For both varieties the total alcohol-soluble substances showed a general drift of falling concentration with fluctuations from November to May and then a rise to a peak value (attained about July 4-11), followed by a rapid fall to harvest. Both the non-sugar and the total sugar components follow definite drifts through the

season, the non-sugar component being, however, three to four times as great as the total sugars from November to May. From May both sugars and non-sugars increased in concentration, the difference between them meanwhile decreasing. After attaining peak values both components decreased rapidly to harvest.

The fluctuations of the percentage of total alcohol-soluble substances during December and January were due to the effects of the frost periods which prevailed during those months. In both varieties the percentage of total sugars responded clearly to low-temperature periods, but a notable difference is observed in the responses of the non-sugar component in the two varieties. In Rivets no effects of the frost period are observed on the percentage of non-sugars, while in Wilhelmina significant rises in the percentage occurred simultaneously with those of the total sugars.

The ear. The percentage of alcohol-soluble substances rose in the ear to a peak value, attained in Rivets on July 4 and in Wilhelmina on June 27, and then fell until harvest. The peak was due solely to the total sugar component, since the non-sugars fell steadily from the time of ear separation. The steady fall of the non-sugar component percentage in the ear indicates that the solutes of this fraction entering the ear from the shoot were speedily transformed into insoluble substances, the rate of entry never rising above the rate of transformation and so no accumulation occurring. This is in contrast with the behaviour of the total sugars where the occurrence of a peak value indicates a rate of formation in the plant and translocation to the ear for some time greater than the rate of utilisation in the ear in starch formation, etc.

Sucrose, glucose and fructose

The three sugars, sucrose, glucose and fructose, are plotted as percentages of the fresh weight of plant material for both varieties throughout the season 1932-3 in Fig. 3 and for the following season in Fig. 4.

In both varieties and for both seasons sucrose was the sugar present in highest percentage in both shoots and separated ears. For the greater part of each season glucose was next highest and fructose the lowest. About 3 weeks or a month before harvest, however, the fructose in the shoots rises in percentage above glucose and remains above it at harvest. In the ears fructose does not increase in percentage appreciably above glucose, but at harvest they are present to approximately the same extent.

Sucrose in the shoot. The sucrose percentage in winter and spring was sensitive to temperature variations, the percentage amounts

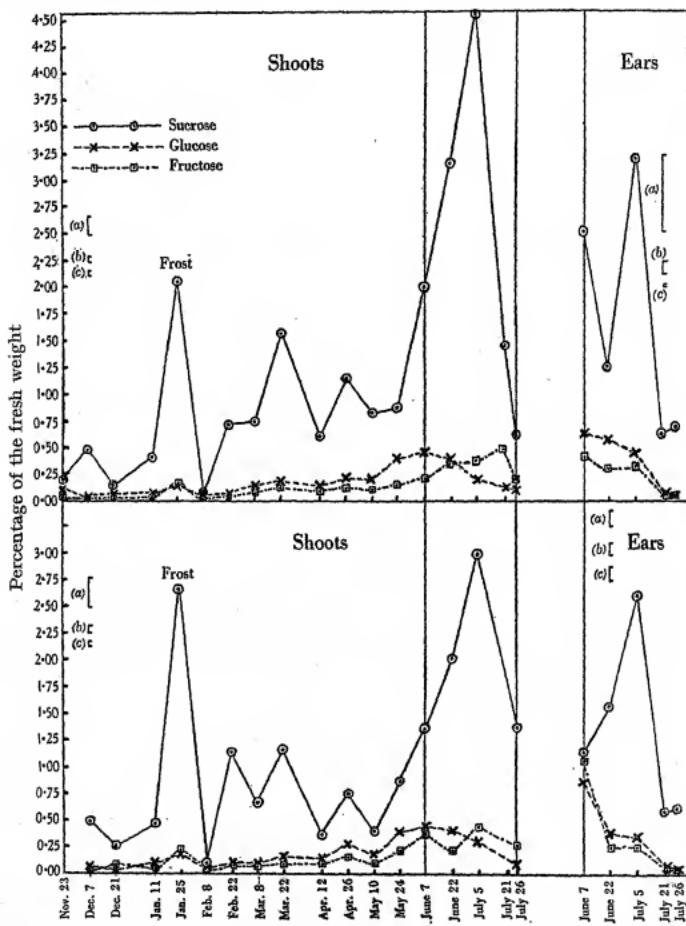


Fig. 3. Drifts of sucrose, glucose and fructose during 1932-3. Top curves are from Rivets and the lower from Wilhelmina. Values for the ears and the shoots with ears removed are shown separately after June 7, 1933. The vertical lines labelled (a), (b), (c) for each set of curves give the minimum significant difference (a) of sucrose, (b) of glucose, (c) of fructose.

increasing with low temperatures and falling with moderate temperatures. This production of high concentrations of sugars by the

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influence of cold was shown to be characteristic of winter-hardy wheat varieties by Akerman and his co-workers (1917). During 1932-3

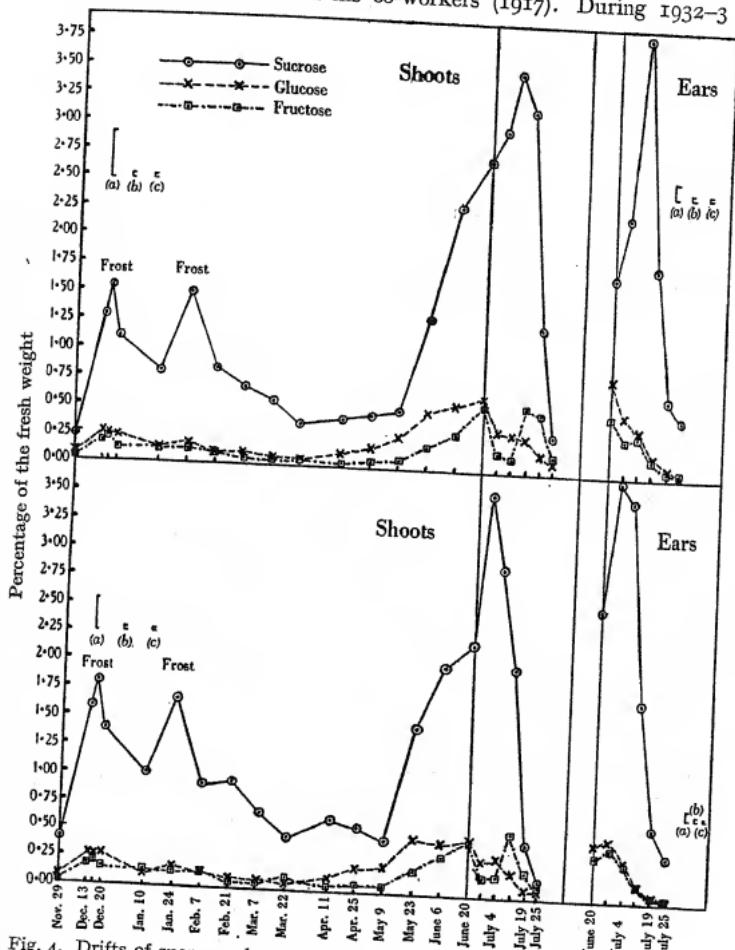


Fig. 4. Drifts of sucrose, glucose and fructose during 1933-4. Top curves are from Rivets and the lower from Wilhelmina. Values for the ears and the vertical lines labelled (a), (b), (c) for each set of curves give the minimum significant differences (a) of sucrose, (b) of glucose, (c) of fructose.

season particularly high percentages of sucrose occurred in both Rivets and Wilhelmina in the samples obtained on January 25 (2.05

and 2·65 per cent. respectively, Fig. 3) and these were followed by very low values (0·09 per cent. for both varieties) in the samples of a fortnight later. The low temperatures on and before January 25 were associated with more than average sun,¹ and so photosynthesis may have continued while utilisation of sugars in respiration, fibre formation and general growth was slowed down, and so sugars, particularly sucrose, piled up in the plant. A period of relatively high temperature for the time of year and little sun followed, during which, presumably, growth and respiration rates increased while photosynthesis was slow, i.e. sugar utilisation was rapid and sugar formation slow, resulting in a low percentage of sugars in the plant.

Two cold spells in December and January, respectively, during the 1933–4 season produced effects on the sucrose content of the two varieties though not to so great an extent as the prolonged cold period of the previous season. Three samples were taken during the week beginning December 13, 1933; the first two coming within the December cold period and the third after the temperature had risen somewhat. In Rivets the sucrose content rose from 0·25 per cent. on November 29 to 1·30 per cent. on December 13 and continued to rise during the cold weather reaching 1·55 per cent. 3 days later (December 16), it then fell to 1·11 per cent. on December 20. Similar values were obtained for Wilhelmina over the same period. It is therefore seen that the percentage of sucrose does not at once rise to its maximal value in cold weather, but can continue to increase if the temperature remains low; it falls as soon as there is a change to warmer conditions. Both December 1933 and January 1934 were, on the whole, cold months, and the sucrose percentage in Rivets and Wilhelmina accordingly remained relatively high throughout this time showing fluctuations with varying temperatures.

The origin of the excess sugar formed during cold periods does not lie in any of the polysaccharides estimated. No diminution in the percentage of taka-diastase hydrolysis products occurred in the January 25 sample of 1933 (Fig. 5), and in the following season the fall in taka-diastase hydrolysis products in Rivets between November 29 and December 13, 1933 (Fig. 6) of 0·54 per cent. was insufficient

¹ The following are the relevant meteorological data from the Cambridge University Farm:

Week beginning	Jan. 1	Jan. 8	Jan. 15	Jan. 22	Jan. 29	Feb. 5	Feb. 12
Mid-temp. of week (°F.)	44·5	37·4	33·9	28·7	40·0	48·3	37·4
Hours of sun in week	10·1	17·0	6·0	33·6	17·4	14·7	45·6

to account for the rise of 1·05 per cent. in the sucrose between the same two dates. The fall in taka-diastase hydrolysis products in both Rivets and Wilhelmina between January 10 and 24, 1934, was very small compared with the sucrose rise. The rise in the sucrose percentage under frost conditions may therefore have been due, to some extent at least, to the rate of production of sucrose by the photosynthetic process exceeding its rate of utilisation in the growth and respiration processes.

From April onwards the sucrose percentage followed a definite drift clearly shown in both varieties in both seasons (Figs. 3 and 4) and little affected by environmental conditions. No effect on the sucrose drift is observed by the inception of tillering which was in early February in each season. From the beginning of April in each season the sucrose rose, at first slowly and with fluctuations, then in May the rate of increase of sucrose content became greater and eventually a peak value was attained in late June or early July. The peak values were: Rivets, July 5, 1933, 4·55 per cent.; Wilhelmina, July 5, 1933, 2·99 per cent.; Rivets, July 4, 1934, 3·53 per cent.; Wilhelmina, June 27, 1934, 3·55 per cent. After the peak value was attained, the sucrose percentage fell continuously to the date of harvest. When the simultaneous drift of the water content is taken into account this sucrose drift is modified but not obliterated. The magnitude of the sucrose content, expressed as a percentage of the dry weight, at its peak value is of the same order as the high values of sucrose (as percentages of the dry weight) produced under low temperature conditions; i.e. approximately the same amount of sucrose per unit weight of dry matter may be present when the temperature is low during winter or early spring as during the developmental drift in early summer. The following values selected from both seasons and the two varieties for the sucrose percentages of the dry matter illustrate this point:

	1933		1934	
	Low tempera- ture value Jan. 25	Peak value July 5	Low tempera- ture value Dec. 16	Peak value July 4
Rivets	11·71	12·52	9·03	8·87
Wilhelmina	10·31	8·78	9·65	9·54 (June 27)

The separation of ears from the plants on June 7, 1933 and on June 20 in 1934 caused no abrupt change in the sucrose curves for the shoots, and analyses of the ears show essential similarity in carbohydrate content with the rest of the plant at the time of separation.

Sucrose in the ear. The drift of sucrose percentage in the ears from the time of their separation followed a similar course to that in the shoot at the same time (Figs. 3 and 4). The sucrose rose¹ during June and in each case attained a peak value on the same date as it was attained in the shoot. The peak value of the sucrose content in the ears was lower than that in the shoot in both varieties in 1933 and not significantly above the shoot values in 1934. Following the peak values, the sucrose content of the ears fell to a relatively low value by harvest, as in the shoots.

Glucose and fructose in the shoot. The glucose and fructose percentages show little change during the winter apart from variations induced by temperature changes. Both increased at low temperatures, the fructose tending to do so more than the glucose (Fig. 3, January frost period). A possible explanation of this may be found in Onslow's (1931) suggestion that γ -fructose is the preferentially respired sugar; a lowering of the respiration rate in cold weather may permit of the accumulation of fructose relative to glucose.

Both sugars increased in percentage amounts from March at first slowly and then from May more rapidly. Glucose in both varieties in 1933 (Fig. 3) rose to a maximum value on June 7 and then fell steadily to a low value on the last sampling date, July 26. Fructose increased also in amount at this time but did not attain its maximum until 4–6 weeks later. The peak values were approximately the same for both glucose and fructose.

During the season 1933–4 (Fig. 4) both glucose and fructose percentages were relatively high during December and January owing to the cold weather. They fell during February and March and then the slow rise of the developmental drift becomes apparent. Glucose increased more rapidly than fructose and in both varieties attained its peak value on June 20 and then fell to relatively low values on June 27 and July 4. The more leisurely fall in percentage to the date of harvest then followed. Fructose also increased from April onwards, but between June 20 and 27 it also fell, then, following low values on July 4, rose to its peak value on July 11, rising above the then falling glucose amount. From July 11 the fructose content fell to harvest.

This sudden fall in percentage by both glucose and fructose between June 20 and 27, 1934, was apparently not related to any sudden change in temperature or illumination but to the breaking of

¹ The fall in the sucrose content of the ears of Rivets between June 7 and 20, 1933, is open to suspicion, as the value for June 7 is based on analyses of very small samples of fresh material: 1 and 4 gm. respectively for duplicates instead of the usual 50 gm.

a period of drought. From the end of the first week in May until June 17 only 0.58 in. of rain had fallen and then during the weeks beginning June 17 and 24 respectively, 0.42 and 0.50 in. fell. This rain had no measurable effect on the water contents of the shoots, so the fall in the sugar percentages was not a simple dilution. It is possible that injection of the leaves by water caused an increased respiration rate with a consequent excessive rate of decomposition of hexoses. If we adopt Onslow's preferential respiration of γ -fructose hypothesis, then the fact that fructose fell to a lower value than the glucose can be used as corroborative evidence of the increased respiration rate explanation of the fall.

In both varieties in both seasons the fructose percentage rises above that of the glucose 2-3 weeks before harvest and remains higher, up to and including the last sampling date. This may, possibly, be considered evidence in favour of the hypothesis of preferential respiration of fructose as the respiration intensity of the ageing tissues of the shoots would be decreasing in this period.

Glucose and fructose in the ears. The percentages of both glucose and fructose fell from the time of separation of the ears until harvest (Figs. 3 and 4). Glucose was present in greater percentage (except in Wilhelmina, June 7, 1933) than fructose at the time of separation but the difference decreased in successive samples until on the last sampling date it was negligible or the fructose content became slightly higher than that of glucose.

Taka-diestase hydrolysis products

The shoot. The reducing sugars (glucose and pentoses) appearing under this heading were estimated after hydrolysis of the alcohol extracted plant residue with taka-diastase and the two fractions (*a*) fermentable (glucose), (*b*) non-fermentable (pentoses), are shown together with the total products in Figs. 5 and 6 for both varieties through the two seasons. The total hydrolysis products fluctuated in amount during the winter and spring months in a manner little correlated with the weather. In Fig. 6 the pronounced fall in the amount of the total polysaccharides in both varieties between November 29 and December 13, 1933, was associated with the rise in sucrose percentage due to frost. The fall was not adequate to account for this sucrose rise, but since the sun record at that time was low (there was no sun on either of the two days preceding December 13) the rate of photosynthesis may not have been sufficient to outbalance a differential effect of low temperature on the hexose-

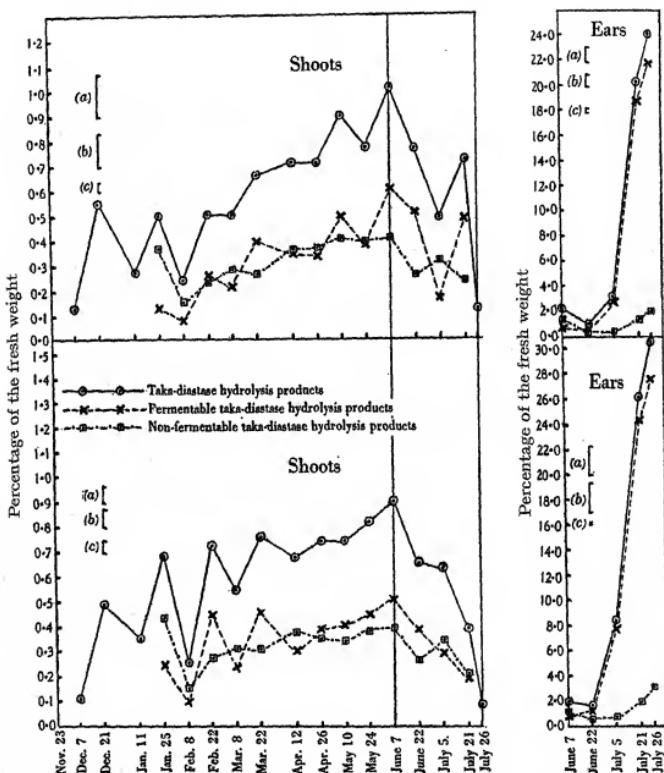


Fig. 5. Drifts of taka-diastase hydrolysis products and the component fermentable and non-fermentable fractions during 1932-3. Top curves are from Rivets and the lower from Wilhelmina. Values for the ears and the shoots with ears removed are shown separately after June 7, 1932. The vertical lines labelled (a), (b), (c) for each set of curves indicate the minimum significant difference (a) of total taka-diastase hydrolysis products, (b) of fermentable taka-diastase hydrolysis products, and (c) of non-fermentable taka-diastase hydrolysis products.

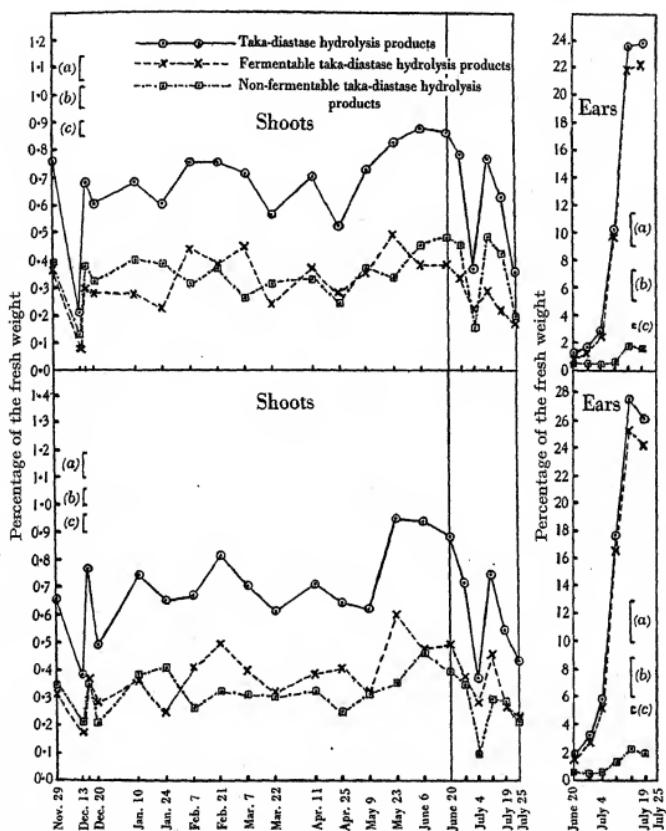


Fig. 6. Drifts of taka-diastase hydrolysis products and the component fermentable and non-fermentable fractions during 1933-4. Top curves are from Rivets and the lower from Wilhelmina. Values for the ears and the shoots with ears removed are shown separately after June 20, 1934. The vertical lines labelled (a), (b), (c) for each set of curves indicate the minimum significant differences (a) of total taka-diastase hydrolysis products, (b) of fermentable taka-diastase hydrolysis products, and (c) of non-fermentable taka-diastase hydrolysis products.

polysaccharide balance. If the rate of condensation of hexoses to polysaccharides fell relative to the rate of hydrolysis of the polysaccharides then a decrease in the amount of the latter would result. The rise in polysaccharides to December 16, occurring in both varieties, followed two days having 5·2 and 5·5 hours of sun each. Suggestions concerning the effects of weather conditions on the total hydrolysable polysaccharide content are tentative, as in general little correlation could be detected.

From spring (April or May) the total polysaccharides rose, reaching peak values at the beginning of June or the end of May in the case of Wilhelmina (1934). The percentage then fell to harvest, but in 1934 in both Rivets and Wilhelmina there was a temporary steep fall between June 27 and July 4 which may be correlated with the low concentration of hexoses present at this time.

The drifts of both the fermentable (glucose-yielding) and the non-fermentable (pentose-yielding) fractions were very similar to those of the total polysaccharides, and the fractions were present in approximately equal amounts in the shoots throughout the season.

The ear. The drifts of the total taka-diastase hydrolysis products and the fermentable and non-fermentable components are shown for the ears alone in Figs. 5 and 6. The large increase which occurred in the percentage of the fermentable hydrolysis products necessitates the use of a scale much smaller ($\frac{1}{20}$ th) than that used for the shoots.

In 1933 (Fig. 5) ears were first separated from the shoots on June 7 while in 1934 (Fig. 6) the separation was first carried out on June 20. There was little change in either variety between the percentages of total taka-diastase hydrolysis products on June 7 and 22 (1933), but after this date in 1933 and after June 20, 1934 (Fig. 6), the percentage increased at first relatively slowly and then, after the first week in July, rapidly. In both seasons the rise was more rapid in Wilhelmina than in Rivets.

The increase in the total hydrolysis products was due almost entirely to the fermentable fraction. Iodine tests showed the complete absence of starch in the June 7 samples, on June 22, 1933, a trace of starch appeared in the ears of Wilhelmina but none in Rivets, while succeeding samples all had much starch in them. In 1934 the first samples (June 20) showed traces of starch in the ears of both varieties, and the following samples all had much starch in them. It is reasonable therefore to regard the fermentable fraction of the hydrolysis products as glucose produced mainly from starch and therefore as giving a measure of the developing starch content of the ears.

The rise in the fermentable fraction of starch content of the ears coincided with the fall in the sucrose content after the peak values attained in the first week in July (Figs. 3 and 4).

Bound and free glucose

The shoot. During the season 1933-4 glycosides were estimated (as glucose liberated on hydrolysis) to obtain estimates of the additional resources of glucose possessed by the plant in bound or combined form. The glycoside glucose, free glucose and total (bound plus free) glucose are plotted for both varieties throughout the season in Fig. 7. The drifts of the total and of the glycoside glucose are similar in most respects for the two varieties.

The total and the bound glucose were both high in December, suggesting that cold weather tends to increase the glycoside content of wheat. The number of determinations of glycosides was too few in January for the effect of low temperatures to appear in this month. The total glucose began to increase in percentage from March and attained ill-defined peak values in both varieties about the end of June or beginning of July. It then fell to the date of harvest. The bound or glycoside glucose percentage increased from the end of March with some fluctuations, reaching peak value on July 4 in Rivets and July 11 in Wilhelmina; it then fell until harvest.

The ear. Total glucose and its components of free and bound glucose fell in percentage from June 20 to harvest, apart from minor fluctuations. The relation between bound and free glucose was closer in the ear than in the shoot. In the shoot during June and early July the rate of formation of glycosides from glucose exceeded their hydrolysis rate. In early July starch formation in the ear began, and presumably glucose was translocated in increasing amounts from the shoot to the ear and there condensed to starch. With decreasing amounts of glucose in the shoot, the hydrolysis rate of the glycosides eventually exceeded the condensation rate of glucose to glycosides, and so about the middle of July the glycosides in the shoot began to fall in amount. The mechanism in the ear is different; the glycoside content remained lower than that of the free glucose to nearly the end of the season and fell in percentage along with the free glucose. The activity of the condensing system in the ripening ear appears to be less great than in the shoot, the rate of hydrolysis of glycosides being greater than their formation rate. The fall in free glucose in the ear must be ascribed to the activity of the condensing mechanism responsible for starch formation from the sugars in the ear.

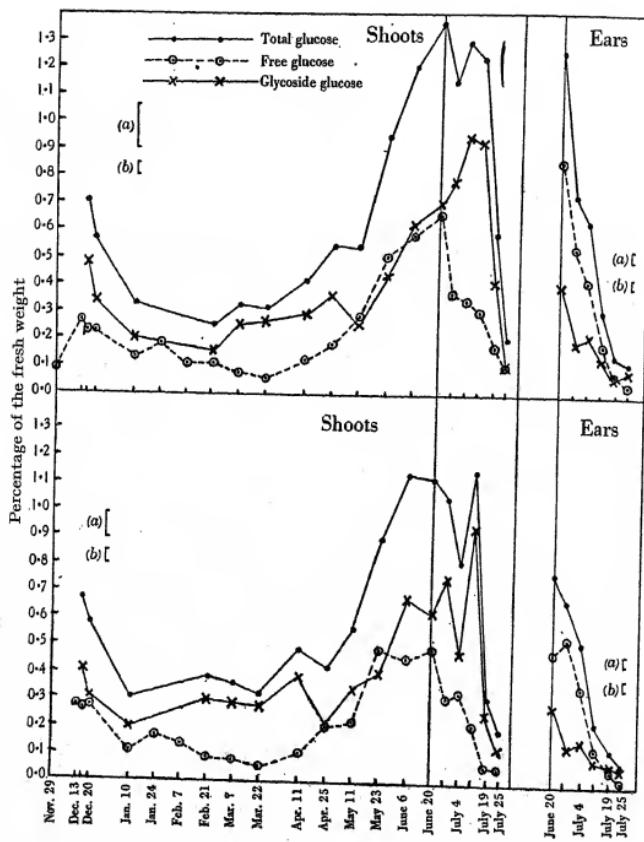


Fig. 7. Drifts of glycoside ("bound") glucose, "free" glucose and total glucose during 1933-4. Top curves are from Rivets and the lower from Wilhelmina. Values for the ears and the shoots with ears removed are shown separately after June 20, 1934. The vertical lines labelled (a), (b) for each set of curves indicate the minimum significant difference (a) of glycoside glucose, (b) of free glucose.

TOTAL AMOUNTS OF DRY MATTER AND OF VARIOUS CARBOHYDRATES
IN RIVETS AND WILHELMINA DURING 1933-4

The necessity for reducing to a minimum the time between sampling and the immersion of the sample in boiling spirit rendered it impossible to count the tillers per sample throughout the season. However, during the season 1933-4 the weight of each sample, consisting of ten 1-ft. row units, was obtained and the amount of each estimated substance per 10-ft. row calculated for that season. This is, probably, a sounder method of presenting such data than of giving the amount per plant or per tiller, since the effects of spacing irregularities are reduced.

The whole plant. The mean dry weights and the amounts of various carbohydrates per 10-ft. row are given in Tables II and III for Rivets and Wilhelmina respectively during 1933-4. The data are presented (*a*) for the whole plants, (*b*) for the ears, (*c*) for the shoots from which the ears were separated.

The dry weight of the plants in both varieties remained low during December and January with little increase, but in February, with the beginning of tillering, it started to rise steadily till April when vigorous growth began. The dry weight increased tenfold in each case between April 11 and 25. The period of vigorous growth continued until early July, and then the stems and leaves decreased in weight while the ears increased at their expense.

Up till the end of March there was little change in the total amount of glucose or fructose; the total amount of each varied between 0.02 to 0.05 gm., but there was no drift; the glucose was usually greater in amount than the fructose. Presumably during this period of little growth the hexose sugars are in a balanced state; their rate of formation by photosynthesis is slow owing to little sun and low temperatures, but their rate of utilisation in growth and respiration is also slow and approximately balances their formation rate. Hence once the initial amount of hexose is present in the plants (by translocation from the grain and photosynthesis) the amount remains approximately the same throughout the early months of winter.

There was an increase in the total amount of sucrose in cold weather; thus in Rivets it rose from 0.021 gm. on November 29 to 0.180 gm. on December 13 and to 0.232 gm. on December 16, falling after the frost period to 0.159 gm. on January 10 and then rising with another period of cold weather to 0.300 gm. on January 24. In

TABLE II

Amounts of dry matter and carbohydrates per 10-ft. row.
Rivets, 1933-4

Date	Total dry matter gm.	Glucose gm.	Fructose gm.	Sucrose gm.	Taka- diastase hydro- lysis products gm.	Ferment- able taka- diastase hydro- lysis products gm.	Glyco- sides gm.
(a) Whole plants							
Nov. 29	1.30	0.007	0.004	0.021	0.066	0.032	—
Dec. 13	2.28	0.037	0.026	0.180	0.030	0.011	—
Dec. 16	2.56	0.034	0.034	0.232	0.102	0.045	0.073
Dec. 20	2.06	0.041	0.023	0.201	0.109	0.051	0.060
1934:							
Jan. 10	3.17	0.025	0.025	0.159	0.132	0.055	0.039
Jan. 24	3.21	0.036	0.029	0.300	0.121	0.045	—
Feb. 7	4.95	0.033	0.036	0.258	0.231	0.134	—
Feb. 21	5.72	0.038	0.023	0.220	0.262	0.133	0.053
Mar. 7	6.33	0.029	0.024	0.226	0.288	0.180	0.104
Mar. 22	7.80	0.028	0.030	0.193	0.293	0.129	0.137
Apr. 11	14.31	0.122	0.043	0.412	0.681	0.354	0.284
Apr. 25	15.70	1.200	0.356	2.940	3.340	1.790	2.290
May 9	21.20	3.230	0.978	5.780	8.210	4.030	2.840
May 23	23.00	5.990	2.560	15.780	9.790	5.810	4.330
June 6	41.40	9.440	5.250	37.800	14.320	6.250	10.110
June 20	53.70	11.180	9.400	43.480	15.270	7.230	11.030
June 27	50.70	5.470	2.780	41.110	12.940	6.480	9.880
July 4	58.80	5.360	2.620	54.000	12.700	9.600	12.200
July 11	71.90	4.280	7.280	44.370	55.400	45.900	10.890
July 19	60.80	1.840	4.390	14.270	12.300	10.7900	3.840
July 25	74.10	1.510	2.330	7.330	14.9700	13.7200	1.810
(b) Ears							
June 20	71.00	1.670	1.020	3.480	2.640	1.600	0.780
June 27	69.00	0.996	0.617	4.230	3.320	2.420	0.357
July 4	102.00	1.160	1.010	10.900	8.160	6.920	0.616
July 11	188.00	0.875	0.743	8.250	46.700	42.700	0.576
July 19	251.00	0.374	0.359	3.380	115.000	106.000	0.344
July 25	322.00	0.236	0.333	3.100	145.100	135.100	0.545
(c) Shoots only							
June 20	466.00	9.510	8.380	40.000	12.630	5.630	10.250
June 27	438.00	4.470	2.160	36.880	9.620	4.060	9.520
July 4	486.00	4.200	1.610	43.100	4.540	2.680	11.580
July 11	531.00	3.460	6.540	36.120	8.700	3.240	10.310
July 19	357.00	1.470	4.030	10.890	5.360	1.860	3.500
July 25	419.00	1.270	1.990	4.230	4.570	2.120	1.260

Wilhelmina the corresponding figures were: November 29, 0.058 gm.; December 13, 0.299 gm.; December 16, 0.280 gm.; January 24, 0.274 gm.

The taka-diestase hydrolysis products fluctuated in amount during December and January but started definitely to increase in February and continued to do so for the rest of the season apart

TABLE III

Amounts of dry matter and carbohydrates per 10-ft. row.
Wilhelmina, 1933-4

Date	Total dry matter gm.	Glucose gm.	Fructose gm.	Sucrose gm.	Taka-diaستase products gm.	Fermentable taka-diaستase hydrolysis products gm.	Glycosides gm.
(a) Whole plants							
1933:							
Nov. 29	2.17	0.012	0.004	0.058	0.095	0.045	—
Dec. 13	3.36	0.052	0.036	0.299	0.073	0.033	—
Dec. 16	2.90	0.041	0.032	0.280	0.121	0.058	0.063
Dec. 20	3.20	0.049	0.027	0.249	0.089	0.051	0.055
(b) Ears							
June 20	77.00	1.760	1.070	3.660	2.780	1.690	0.823
June 27	89.00	1.370	0.851	5.830	4.570	3.320	0.492
July 4	146.00	1.660	1.440	15.580	11.670	9.860	0.880
July 11	225.00	0.850	0.775	8.620	48.700	45.300	0.601
July 19	325.00	0.441	0.424	3.990	135.800	125.300	0.406
July 25	291.00	0.206	0.292	2.720	127.200	118.300	0.477
(c) Shoots only							
June 20	429.00	6.150	5.960	27.700	11.020	6.140	7.740
June 27	434.00	3.620	1.940	41.300	8.350	4.320	8.740
July 4	473.00	3.800	2.130	33.290	4.280	3.220	5.460
July 11	425.00	1.850	4.870	17.490	6.510	3.940	8.120
July 19	392.00	0.534	1.760	3.920	4.550	2.140	2.080
July 25	318.00	0.478	0.913	1.230	3.800	1.980	1.200

from minor fluctuations (the same is true of the fermentable fraction of the total hydrolysis products). During the winter months there appears to be little indication of a relation between free glucose and glycoside glucose.

The total amounts of glucose, fructose, sucrose and total taka-diaستase hydrolysis products are plotted for both varieties in Fig. 8

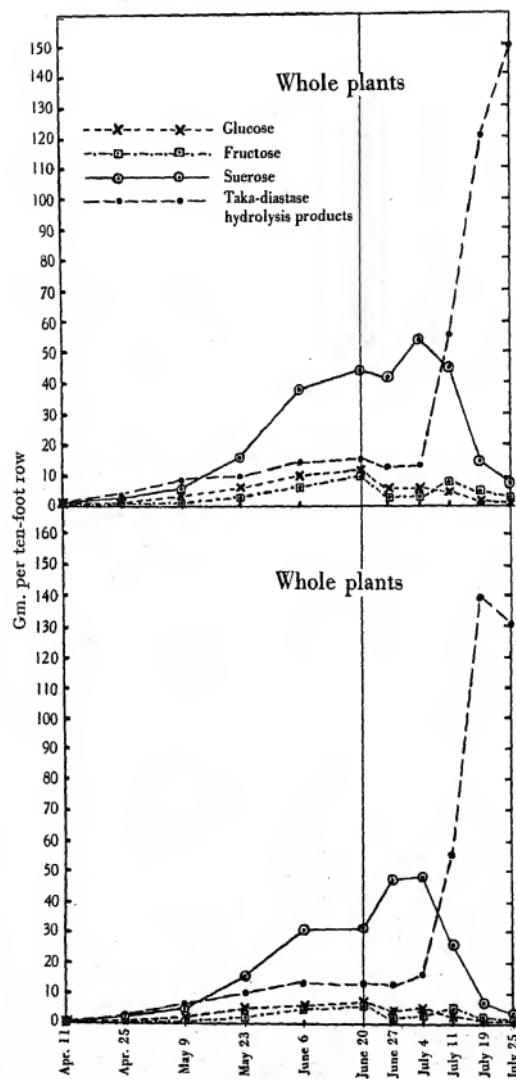
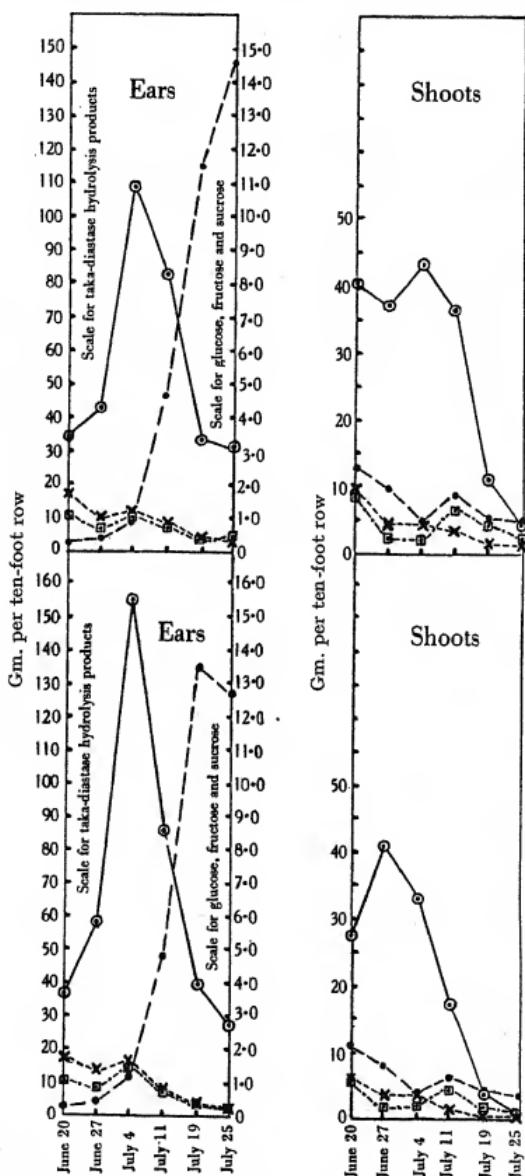


Fig. 8. Progress of carbohydrate accumulation per 10-ft. row of wheat during 1934. The amounts of glucose, fructose, sucrose and taka-diastase hydrolysis products present at various times are shown from April 11 to July 25.



Top curves are from Rivets and the lower from Wilhelmina. Values for the shoot plus ears, ears, and shoots with ears removed are shown separately after June 20, 1934.

from April to the end of the season. From the beginning of April each substance began to accumulate in the plant. Sucrose increased in total amount in each variety until July 4, at which date approximately 50 gm. per 10-ft. row was present; the amount fell on subsequent dates and simultaneously the taka-diastase hydrolysis products (consisting mostly of the fermentable fraction) rose rapidly. The rapid increase of this was due almost entirely to the piling up of starch in the ears [see (b) and (c) in Tables II and III]. Glucose and fructose rose to lesser maxima than the sucrose, and their amounts after June 20 were affected by the break in the drought prevailing till then. The glucose maximum was reached on June 20 in each variety, and then the amount fell till harvest. Fructose reached its greatest amount on the same date, but had the subsequent fall due to breaking of the drought not occurred, it is more than probable that it would have further increased, reaching its maximum later than glucose. This conclusion is reached after examination of the relations of the percentage amounts of glucose and fructose in the plants during the two seasons investigated and also from the fact that later (July 11) in each variety the fructose amount rises above that of glucose. There was, then, a sequence of maxima in the amounts of sugars present: first, glucose rose to a maximum towards the end of June at approximately the time of ear emergence; the subsequent fall was, at first, probably due to utilisation in growth which was vigorous at this time and later to condensation to starch in the ear. Sucrose rose to its high maximum amount after glucose and its accumulation was, presumably, due to a high rate of photosynthesis associated with a very high critical concentration for starch formation. About the beginning of July a change must have occurred in the carbohydrate condensation-hydrolysis mechanism of the ear resulting in a relatively low value for the critical concentration, with the result that the amount of sucrose began to fall. The total fall in the amount of sucrose from its peak value till harvest accounts for less than half the polysaccharides piled up in the ears; the remainder must have been derived from the direct translocation of the products of photosynthesis to the ear and their condensation there during this period of decreasing sucrose amount.

The rise in the amount of fructose from April onwards was most probably due to the high photosynthetic activity of the plant, a balance being maintained between the sugars. The lower amount of fructose at any time was, perhaps, due to the preferential respiration of γ -fructose, a slow rate of conversion of glucose to fructose

preventing the latter from equalling the former. The eventual rise in the amount of fructose above that of glucose may be ascribed either to a lowered respiration rate in the plant or to the existence of a higher value for the critical concentration for fructose than for glucose, the fructose having to be converted into glucose before condensation.

The total amounts of glycoside glucose and free glucose in the whole plants from April 25 to the end of the season are shown for both varieties in Fig. 9. There is clearly a relationship between the free glucose and glucose bound in the form of glycosides, the latter increasing as glucose increased; but the approximate curves drawn through the points show that in each variety the peak value for glycoside glucose was later than that for free glucose. That is, when the rate of utilisation of glucose exceeded its rate of formation and the amount of glucose began to fall, the glycoside amount was not immediately decreased but its rate of formation decreased, and when the free glucose had fallen to a particular concentration the rate of hydrolysis of glycosides became greater than their rate of formation, the fall in glycoside amount then beginning. This is explicable on the assumption that the velocity constants for the condensation of glucose to glycosides are, on the whole, greater than the velocity constants for the hydrolysis of glycosides to glucose, etc. The glucose-containing glycosides of the plant can be regarded as a reservoir of glucose which is drawn upon by the plant in times of large sugar requirement.

The ears. The amount of dry matter in the ears increased, apart from small fluctuations, from the time of first separation of the ears till harvest [Tables II (b) and III (b)]. The amount of sucrose (Fig. 8) rose in both Rivets and Wilhelmina to a maximum value on July 4 and then fell. The rise in the sucrose up to that date was due to the piling up of sucrose in the whole plant (not especially the ear) caused by the high value of the critical concentration for starch formation. There was little increase in the polysaccharide content of the ears until after July 4. About this date some change, perhaps due to decreasing water content, took place within the ears causing the value of the critical concentration to be lowered. Sucrose was then converted, by some mechanism unknown, into starch, and fell in amount, although fresh supplies of sucrose would have been continually arriving in the ear from the supplies created in the rest of the plant by photosynthesis.

The amounts of glucose and fructose in the ears both fell from the time of first separation of the ears, though in both varieties both

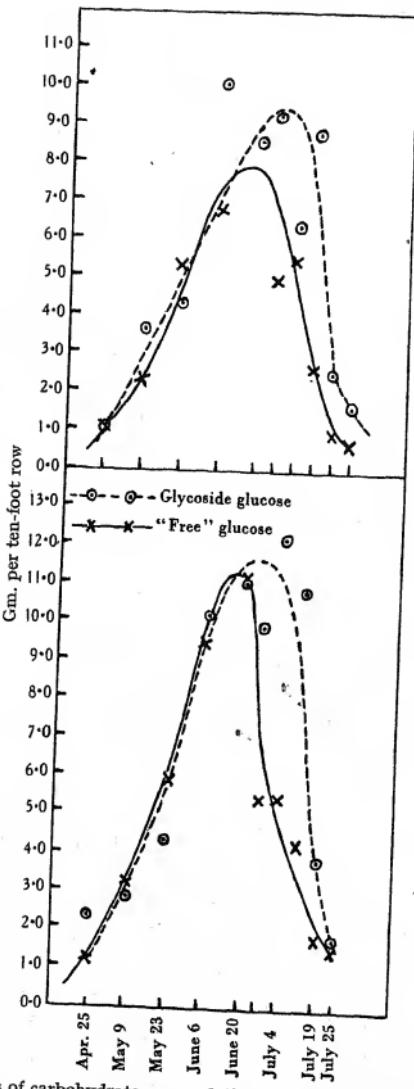


Fig. 9. Progress of carbohydrate accumulation per 10-ft. row of wheat during 1934. The amounts of glycoside ("bound") glucose and "free" glucose present in shoots plus ears at various times are shown from April 25 to July 25. The top curves in the figure are from Wilhelmina, those below are from Rivets.

sugars showed a rise between June 27 and July 4 causing an interruption in the steady fall. Glucose was greater in amount in the ears than fructose on June 20, but the difference between the amounts of the sugars decreased until at harvest fructose was slightly in excess of glucose.

Earless shoots. From June 20 the ears were separated from the shoots for separate estimation of carbohydrates. The dry weight of the shoots increased up till July 11 in Rivets [Table II (c)] and up till July 4 in Wilhelmina [Table III (c)], and after these dates decreased in amount due to losses by translocation of sugars to the ears to form starch, cellulose, etc., and by respiration; also the activity of the photosynthetic mechanism was, presumably, decreasing with the drying out of the tissues.

The amounts of the carbohydrates in the shoots alone are given in Tables II (c) and III (c) and are plotted in Fig. 8. Apart from the taka-diastase hydrolysis products the drifts of the carbohydrates in the shoots are similar to those in the whole plants over the same period. The taka-diastase hydrolysis products (including apparently no true starch) fell with some fluctuations to the date of harvest.

RELATION BETWEEN SUCROSE CONTENT OF WHEAT AND THE TEMPERATURE DURING WINTER AND SPRING

In both seasons in both varieties of wheat periods of low temperature during the winter and spring caused the cane sugar concentration to rise. The source of this additional sucrose in cold weather must be left as a subject for direct experimental investigation, since from the present data it is not possible to settle whether it results from the slowing down of growth processes causing accumulation of the products of photosynthesis in the cell vacuoles or whether it has some unknown origin in substances stored in the plant. There is little evidence in the data of decreasing amounts of polysaccharides associated with rising sucrose content, such as would be expected if the low temperatures were affecting the polysaccharide-sugar balance.

To obtain a more quantitative outlook on the sucrose-temperature relation, various correlation coefficients are presented in Table IV. For both seasons for both varieties the correlation coefficients are given between sucrose expressed as percentage of the fresh weight and of the dry weight, and the mid-temperature of the day preceding sampling. In two cases the coefficient is given for the sucrose content and the mid-temperature of the week preceding that in which the sample was taken. In one case also the sucrose is expressed as a

TABLE IV

Relation between sucrose content of wheat and the temperature during winter and spring

Variety	Season	Period	Sucrose content expressed as % fresh wt.	Mid-temp. of preceding day	Correlation coefficient
Rivets	1932-3	Nov. 23-Mar. 22	% fresh wt.	"	-0.62
Wilhelmina	"	Dec. 7-Mar. 22	"	"	-0.75
Rivets	"	Nov. 29-Mar. 22	% dry wt.	"	-0.45
Wilhelmina	"	Dec. 7-Mar. 22	"	"	-0.72
Rivets	1933-4	Nov. 26-Apr. 15	% fresh wt.	"	-0.65
Wilhelmina	"	"	"	"	-0.72
Rivets	"	"	% dry wt.	"	-0.69
Wilhelmina	"	"	"	"	-0.77
"	"	"	% residual dry wt.	"	-0.77
Rivets	"	"	% fresh wt.	"	-0.66*
"	"	"	% of water content	"	-0.63
Wilhelmina	1932-3	Dec. 7-Mar. 22	% fresh wt.	Previous week	-0.59
"	1933-4	Nov. 26-Apr. 15	% dry wt.	"	-0.69

* Partial correlation coefficient, effect of hours of sun eliminated.

percentage of the water content and in another of the residual dry weight of the plant. The values of the correlation coefficients agree well for the two seasons; thus the values for Rivets for 1932-3 and 1933-4 respectively are -0.62, -0.65, and for Wilhelmina -0.75, -0.72, expressing sucrose as a percentage of the fresh weight in each case. There is little difference in the values of the correlation coefficients whether sucrose is expressed as a percentage of the fresh weight, dry weight, water content or residual dry matter, and this is explicable by the little change observed in water contents of the plants (Fig. 1) during periods of low temperatures.

The correlation between sucrose content was higher with the temperature of the day previous to sampling than with that of the week previous (for Wilhelmina 1933-4 the values are -0.77, -0.69 respectively, sucrose expressed as percentage of the dry weight), but the differences are not statistically significant. However, a fairly slow adjustment of the sucrose concentration during a low-temperature period seems to be indicated rather than a rapid one.

Although the concentrations of the hexose sugars were slightly affected by low temperatures in the same direction as that of sucrose the effect on sucrose is so much greater that the correlation of the

ratio $\frac{\text{sucrose}}{\text{hexose}}$ with temperature is very little different from that of sucrose alone. In Rivets (1933-4) the correlation coefficient for the ratio was -0.56 (both components of the ratio expressed as percentages of the fresh weight), while the corresponding coefficient for sucrose was -0.65.

The effect of the amount of incident sun on the sucrose content during the winter and spring was very small. The partial correlation coefficient of sucrose content in Rivets (1933-4) with the mid-temperature of the preceding day eliminating the effect of hours of sun was -0.66 compared with -0.65 for the ordinary coefficient. The actual value for the correlation coefficient for sucrose (as percent. fresh weight) and hours of sun on the preceding day was 0.25. The temperature is very clearly the major external factor controlling sucrose concentration at this time of the season.

INTERVARIETAL DIFFERENCES IN RESPECT OF CARBOHYDRATE CONTENT

The major reason for selecting the two particular varieties of wheat used in this developmental study of the carbohydrate metabolism was to determine any differences in carbohydrate behaviour between two varieties differing considerably morphologically and cytologically.

The carbohydrate contents of the two varieties were compared by determining the ratio of the percentage of each carbohydrate in Wilhelmina to that in Rivets for each sampling time. The mean value of the ratio for each carbohydrate over the whole season was calculated for each season and its difference from unity noted. The significance of this difference was then tested by comparing it with the standard error of the mean ratio (calculated from the estimated standard deviations and Fisher's table of t). A difference greater than twice the standard error was adopted as significant ($P=0.05$ level of significance), and the results are given in Table V. This treatment was adopted with the shoots only, as there were too few values for the ears to allow statistical treatment. The right-hand half of Table V gives the values for the ratios and the tests of the significance of their differences from unity up till the time of ear emergence only, to show whether greater or less differences were to be observed between the varieties during the period when the sugar concentrations were especially sensitive to temperature variations than over the whole season.

TABLE V
Intervarietal differences—shoots. (Each estimated constituent expressed as percentage of the fresh weight)

Whole season		Till time of ear emergence		
Mean ratio Wilhelmina Rivets	s.e. of mean	Diff. of mean ratio from unity	Sig. or not sig. at $P=0.05$	Mean ratio Wilhelmina Rivets
1933-4:				
Glucose	0.890	0.012	-0.110	Sig. R > W
Fructose	1.001	0.064	+0.001	Not sig.
Sucrose	1.064	0.070	+0.064	Not sig.
Taka-diastase	1.042	0.044	+0.042	Not sig.
hydrolysis products				
Glycosides	0.993	0.072	-0.007	Not sig.
1932-3:				
Glucose	1.06	0.08	+0.06	Not sig.
Fructose	1.23	0.14	+0.23	Not sig.
Sucrose	1.02	0.12	+0.02	Not sig.
Taka-diastase				
hydrolysis products	1.04	0.05	+0.04	Not sig.

TABLE VI

Intervarietal differences—ears. (Ratio $\frac{\text{Wilhelmina}}{\text{Rivets}}$ for each estimated constituent expressed as percentage of the fresh weight)

Date	Total alcohol soluble solids	Total reducing sugars	Glucose	Fructose	Sucrose	Taka-diastase hydrolysis products		Non-fermentable taka-diastase hydrolysis products	Glycosides
						Fermentable taka-diastase hydrolysis products	Non-fermentable taka-diastase hydrolysis products		
1933	June 7	1.700	1.451	2.584	0.457	0.805	0.936	0.734	—
	June 22	—	0.751	0.687	1.309	1.361	0.998	0.832	—
	July 5	—	0.802	0.787	0.844	2.518	2.658	1.516	—
	July 21	—	0.940	0.943	0.955	1.281	1.291	1.197	—
	July 26	—	0.709	0.619	0.814	1.271	1.266	1.409	—
1934	June 20	1.022	0.635	0.563	0.757	1.439	1.416	1.819	0.800
	June 27	1.211	1.145	1.386	1.624	1.811	2.139	0.935	0.719
	July 4	0.874	0.884	0.858	0.912	0.908	2.051	2.181	0.721
	July 11	0.877	0.792	0.730	0.855	0.963	1.712	1.709	0.755
	July 19	0.893	0.663	0.662	0.633	0.929	1.169	1.158	0.810
	July 25	0.818	0.532	0.385	0.636	0.770	1.099	1.212	0.719

For 1932-3 not one of the differences between the varieties was significant. With the more numerous observations and improved technique of 1933-4 (use of parallel plots with varieties alternating, instead of separate fields) the sampling error was reduced and some differences became significant. Glucose was slightly higher in percentage in Rivets than in Wilhelmina according to the value for the ratio for the whole season 1933-4, but not according to the value for the winter and spring only. The difference evidently occurred after the time of ear emergence in June and was sufficiently large to influence the ratio and make it significant for the whole season. The only remaining ratio to which full significance was given was that for sucrose up till the time of ear separation in 1933-4. The value for taka-diastase hydrolysis products over the same period lies just on the adopted level of significance and is therefore doubtful in the absence of further data. The higher concentration of sucrose in Wilhelmina over that in Rivets confirms the impression gained concerning the respective sensitivities of the sucrose concentrations of the two varieties to temperature from examination of the drifts of percentage content of sucrose in the plants through the winter and spring. The period over which the sucrose of Wilhelmina is significantly higher than that of Rivets includes the cold-weather periods during which the concentration of sucrose rose higher in Wilhelmina than in Rivets.

Although not subjected to statistical treatment it is possible to draw some conclusions from the ratios of the carbohydrates in the ears, and these ratios are therefore set out in Table VI. The ratios of both sucrose and taka-diastase hydrolysis products in both seasons rose to a peak value and then fell. The cause of this drift is to be found in the time relationships of the developmental drifts of sucrose and polysaccharides in the ears (Figs. 3, 4, 5 and 6). In each case the earlier values of sucrose content were higher in Wilhelmina than in Rivets and at first rose more quickly to the peak value (in 1933-4 the peak in Rivets was not attained until 1 week later than in Wilhelmina), but after the peak the sucrose percentage in Wilhelmina fell rather more rapidly than in Rivets, thus giving decreasing values for the ratio. Since this developmental drift is, in its late stages at least, associated with the ripening of the ear, the drift in the ratios may be ascribed to the quicker ripening of Wilhelmina ears. The drift in the taka-diastase hydrolysis products ratio was also due to the time differences in the developmental drifts in the ears (Figs. 5 and 6). In Wilhelmina in both seasons the rise in polysaccharide content in

the ear during the first 2-3 weeks after ear separation was greater than in Rivets. After approximately the first week in July polysaccharides started to pile up in Rivets ears even more quickly than in Wilhelmina, so the value of the ratio then ceased to rise and after this time fell. The ripening of Wilhelmina ears viewed as polysaccharide deposition proceeded more rapidly in the earlier stages than Rivets, but the latter ripened more quickly in the later stages, though the final total amount of polysaccharide was lower in Rivets in each season.

The values of the ratios for glucose and fructose showed the same rise and fall correlated with different ripening rates of the ears in 1934, but not so clearly in the 1932-3 season (Table VI).

SUMMARY AND DISCUSSION

The percentage amounts of various carbohydrates in the two varieties (Rivets and Wilhelmina) of wheat plants collected at sunrise through both seasons investigated showed well-defined developmental drifts. During winter and early spring the drifts of some of the carbohydrates, particularly the sugars, were somewhat obscured by the effects induced by varying environmental conditions, particularly temperature changes, but it has been possible to obtain a clear picture of the drifts throughout the season and to observe the nature of the complications caused by weather factors.

The sugars occurred in the following order of percentage amounts: sucrose, glucose and fructose. In the absence of considerable weather fluctuations, little change took place in their amounts during winter and spring, but towards the end of spring they began to increase and eventually rose to peak values, attained at different times but in the same sequence each season. Glucose rose to its peak value about the time of ear emergence; sucrose, always the dominant sugar, rose to about 3-4 per cent. of the fresh weight about a fortnight later; and fructose reached its peak about the same time or a little later than sucrose, and in doing so rose above glucose and remained above it at the time of harvest. It is suggested that this sequence of maxima of sugar concentrations in early summer is due to a high rate of photosynthesis giving high concentrations of sugars¹ and the

¹ A contributing cause of the increasing sucrose content of the entire wheat plant in early summer may possibly be found in the effect of desiccation on the polysaccharide-sucrose balance as observed by de Wolff (1926) in the case of the starch-sucrose balance in the potato, in which sucrose accumulates as the water content is lowered. It is proposed to investigate the relation between sucrose and water contents of wheat tissues at a later date.

existence of high values for the critical concentrations of sugars for starch formation in the ears. Glucose falls in concentration after ear emergence because, perhaps, of the vigorous growth taking place at this time. Then early in July a change occurs in the starch-sugar mechanism of the ears, resulting in low values in the ears for the critical concentrations of sugars for starch formation, and cane-sugar is, in some way, converted into starch and its concentration in the whole plant begins to fall. Starch is also laid down continuously from the products of photosynthesis, since the total loss of sucrose by the whole plant from the time of maximum sucrose content (the time when starch deposition begins) to the end of the season accounts for less than half the amount of starch formed in the ears. That the low value of the fructose concentration during the early part of the season is due to fructose providing the substrate for respiration is a tentative suggestion based on Onslow's hypothesis that γ -fructose is the preferentially respired sugar. Towards the end of the season a falling respiration rate might result in a rise in fructose concentration as observed and the final fall be determined by the drain on all the sugars imposed by the activity of the sugar condensing systems in the ears.

Both varieties of wheat responded to low-temperature conditions by increases in the concentrations of sugars, particularly cane-sugar. The origin of the excess sugar formed is uncertain but did not appear to be derived from the polysaccharides estimated by taka-diastase hydrolysis. There was a fairly high negative correlation between the sucrose content and temperature during winter and early spring and a very small positive correlation with hours of sun on the day preceding sampling. These results support the view that at least one method adopted by winter-hardy plants to combat frost is that of raising the suction pressure of their cells by increasing their content of soluble sugars—particularly sucrose—thus resisting water loss and the resultant denaturing of proteins.

There was no starch (apart from occasional doubtful traces in the guard cells) in the stems or leaves during the whole of either season, but glucose-yielding and pentose-yielding polysaccharides were present in approximately equal amounts and showed little change in amount till the end of the season when both fell; starch was definitely formed only in the ears, and even there was not present until a week or a fortnight after ear emergence.

In the shoots there was a fairly close similarity in the drifts of bound (glycoside) and free glucose, but the former rose to a peak

value towards the end of the season later than free glucose. It is suggested that this was due to a high value of the velocity constant for the condensing direction of the glucose-glycoside reaction relative to the velocity constant for glycoside hydrolysis. In the ripening ears glycosidic glucose was present in lower amount than free glucose; both fell with ripening, but the bound glucose fell in a smooth curve and eventually was slightly above the glucose, which fell in an approximately straight line in relation with time.

The growth of both varieties in terms of dry weight per 10-ft. row samples was little during winter and early spring, and little change in carbohydrate content occurred over the same period apart from increases in the total amount of sucrose during low-temperature periods. Activity started at the beginning of April when the dry weight began to increase and carbohydrates to pile up within the plants. The presentation of the total amounts of carbohydrates at various times during the season gives a picture remarkably similar to that given by the drifts of the percentage amounts. Sucrose increased in total amount in the whole plant and in the shoots and ears respectively till early July and then fell, accumulation of polysaccharides in large amount in the ears starting with the onset of falling sucrose amount. Glucose and fructose amounts rose to successive peak values in the shoots and were influenced by the rain after drought in June 1934 in the same direction as their percentage amounts. In the ears the total amounts of glucose and fructose fell with ripening, the difference between them decreasing till at harvest they were present in approximately equal amounts. Total polysaccharides accumulated in ears only, in the shoots the amount fell from the time of ear emergence to harvest. The glycoside glucose reached its maximum amount in the plant about a week after the maximum of free glucose, probably due to a higher value for the velocity constant for glucose condensation than for that of glycoside hydrolysis. The fall in the glycoside content following the fall in free glucose suggests that glycosides may be regarded as reservoirs of glucose which can be drawn upon by the plant in times of large sugar requirements. The dry weight of the shoots per 10-ft. row began to fall from early July with the inception of starch accumulation and increasing dry weight of the ears.

Comparison of the carbohydrate contents of the two varieties, Rivets and Wilhelmina, over two seasons yields the interesting conclusion that there is practically no significant difference between them in this respect. In 1932-3, when separate fields were used for

sampling, no significant difference was obtained for the carbohydrates of the shoots. Using parallel plots with the varieties sown alternately in 1933-4 gave a smaller sampling error, and a significant difference in sucrose content till the time of ear emergence was obtained, though not for the whole season. This confirmed that Wilhelmina (significantly greater sucrose content) was more sensitive in its sucrose reaction to low temperatures than Rivets. Statistical treatment of the values for the ears was not attempted, but drifts in the values of the ratios of the various carbohydrates in Wilhelmina to those in Rivets were interpreted as due to differences in the rates of ripening of the respective ears. The drifts in the ears of the two varieties were similar but had different time relationships; the ears of Wilhelmina start to ripen more rapidly than those of Rivets, but the latter after a lag period ripen more rapidly and tend to catch up with those of Wilhelmina.

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ON THE STRUCTURE OF THE CONE
SCALES OF *LEPIDOSTROBUS*

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(With Plate III)

IN a set of six serial sections primarily designed to exhibit the structure of a cone of *Sphenophyllum Dawsonii* occur a number of foliar organs having such thick-walled cells that these organs form a conspicuous feature of the slides. Further study has borne out the early opinion that these foliar organs present certain features of novelty, and may clear up some points regarding the lepidodendroid cone, so an account of them has seemed worth putting on record.

These slides were prepared by Mr James Lomax, who has furnished the following data: "Loc. Upper Foot Mine, Shore, Littleborough, Lancashire." The state of preservation of this material is for the most part admirable, even certain thin-walled cells of the vascular area being preserved in some of the sections. The light brown colour of the cell walls appears to indicate rapid penetration of the "petrifying" liquid.

The general features are brought out in Pl. III, figs. 1 and 2, which show the exceedingly simple shape of these objects. They are almost flat, although some sections indicate a slight concavo-convex shape, i.e. the scales were very slightly trough-shaped. No projecting midrib or lateral grooves have been found, but the organs were thickest at the middle, tapering gradually right and left to a very thin margin. Measurements of the thickness of nineteen scales were made at the region of the single central vascular bundle. The thinnest scale measured 0.45 mm. and the thickest 0.90 mm., with an average of 0.67 mm. Sections extending from midregion to edge of a scale were scarce, but seven half-scales were measured, the minimum width being 9.18 mm., the maximum 20.5 mm., and average 13.07 mm., all being calculated for width of a whole scale. The length of a slightly slanting longitudinal section (broken off at each end) was 32 mm. The similarity of the various transverse sections indicates that the scales were lanceolate, and the dimensions clearly show that they were not acicular.

The plan of the scale as seen in transverse section (Pl. III, fig. 2) shows a single central bundle, and at a short distance right and left

from this a pair of cavities extending parallel to the surface. As may be seen in Pl. III, figs. 2 and 4, the mesophyll cells are approximately circular in transverse view, varying from diameter 50μ in the middle region to 17μ near the epidermis. Longitudinal sections show that the mesophyll cells were somewhat longer than broad (Pl. III, fig. 3). Nothing approaching a palisade can be seen, and there is no thickened hypodermis, such as is frequent in leaves and cone scales of some species of *Lepidodendron*. Intercellular spaces are rather small. The thickness of the cell walls is a prominent feature, as has already been mentioned, and is in strong contrast with the appearance of certain leaf sections which are frequent in the slides. Transverse sections do not yield a clear picture of the epidermis. This may be due partly to attrition while the material was collecting before petrifaction, but certain longitudinal sections suggest another explanation; Pl. III, fig. 3 shows that many of the epidermal cells were provided with short, obliquely directed, pointed processes, giving the surface of the scale a somewhat roof-like appearance. It is a notable fact that no stomata have been observed, nor have plastids been distinguished in the cells.

The general features of the vascular bundle are shown in Pl. III, fig. 4. A uniform feature, useful in orientation, is the dark-coloured area having the shape of a broad U in transverse section, the outer or convex side of the U lying toward what we interpret as the abaxial surface of the scale. This interpretation is based partly on the slight curvature of the scale, but is borne out by the *Sigillaria* leaf of Pl. III, fig. 6, which will be seen to present a similar dark area, here placed toward the abaxial keel or midrib. The cells of the U-shaped mass are elongated lengthwise, with nearly transverse end-walls. The dark colour appears to be due to an opaque deposit rather than to thickness of the walls themselves. Inside the U is a mass of thin-walled cells which in longitudinal section are found to be much elongated. Lying adaxial to this mass, in a central position, is the vascular bundle, consisting of xylem flattened parallel to the surface of the scale, and surrounded by delicate tissue which, however, is preserved in some of the sections, and appears to constitute the phloem (*wide infra*). Surrounding the vascular bundle and U-shaped mass is an extensive "transfusion sheath", mostly decayed on account of its delicacy, but represented by a few tracheids or by a large group according to the condition of preservation. It will be seen that this layer was well developed on the flanks of the bundle and was quite thin above and below the bundle. Pl. III, fig. 5 brings out

the characters of the walls of the various tissues as seen in longitudinal section. The tracheids making up the xylem are narrow and show spiral or scalariform markings, while the transfusion tracheids display the same general pattern, but the thickenings are very delicate and more remote from one another than in the xylem. Moreover, the transfusion cells are much wider than are the corresponding elements of the xylem. None of the sections show a difference between proto- and metaxylem.

The identity of these organs may now be considered. Although the series of sections was designed to illustrate the structure of a *Sphenophyllum* cone, there is no reason for entertaining the idea that the organs here described represent either leaves or cone scales of *Sphenophyllum*. The sections exhibit the usual jumble of unrelated forms found in a coal ball. The single bundle and the paired cavities suggest a lepidodendroid affinity, while the absence of a midrib, of lateral sulci, of any appearance of palisade or other sort of chlorophyll tissue, renders it very doubtful that these organs can be leaves. On the contrary, the flat shape, slightly curved, and the stout cell walls would better suit the free terminal region of a cone scale. Fortunately transverse sections of several examples of *Lepidostrobus* have been available for comparison, and it soon appeared that in these the distal, nearly vertical region of the cone scales presented a structure corresponding with the organs under consideration. Pl. III, fig. 7, from the outer region of a cone labelled "Lepidostrobus. Loc. Shore, Littleborough, Lancashire", shows the two lateral cavities and a single vascular bundle. Under higher magnification the bundle exhibits a flat group of tracheids and the U-shaped dark mass lying abaxial to the xylem, with a limited amount of transfusion tissue, but the state of preservation does not warrant presenting a high-power photograph. In all but shape this sporophyll tip corresponds with our scales, and has about the shape of the cone scales shown by Maslen (1899) in his notable paper on *Lepidostrobus* (Pl. 37, fig. 16). A scale from another cone (also from Shore, Littleborough) shows the two lateral cavities mostly filled with soft tissue in a more or less disorganised condition (Pl. III, fig. 8). It seems entirely probable that these decaying masses represent the tissue which originally made up the parichnos strands. It would thus appear that the cavities originated in a lysigenous manner.

Maslen, however, represents neither parichnos strands nor lateral cavities in his drawing of the distal part of a scale. One of his drawings (Pl. 37, fig. 18) moreover represents the vascular bundle

as mesarch collateral. It may be suggested that pressure closed up the narrow lateral cavities so that they escaped notice. Moreover different species of *Lepidostrobus* may perhaps differ in the distance to which the parichnos strands extend. Whether the bundle was collateral or concentric depends partly on the interpretation of the mass lying abaxial to the xylem. This U-shaped mass and enclosed tissue correspond exactly in position to that described by Bertrand (1891, Fig. 80) accompanying the leaf traces of *Lepidodendron Harringtonii*, and by him regarded as secretory in function ("l'appareil laticifère"). Seward (1899) adopts this view in figuring the leaf traces of *Lepidophloios fuliginosus*. With our Pl. III, fig. 4, may be compared the photograph (fig. 6) of the central region of a finely preserved leaf of *Sigillaria* (labelled "Loc. Upper Foot Mine, Shore, Littleborough, Lancashire"). The xylem in this instance is clearly diarch, and is surrounded by a layer of thin-walled cells; the U-shaped mass (secretory?) is evident, and the whole is surrounded by a layer of reticulated tracheids which constitute the transfusion tissue. Following the statement of Renault (1889) concerning the foliar bundle of *Lepidodendron rhodumneense*, we may regard the bundle of Fig. 6 as diarch concentric; the bundle of our "unknown" corresponds with this in all respects except that in our Pl. III, fig. 4 it is not possible to distinguish protoxylem. It appears clear from numerous sections that the sporophyll traces began as collateral strands; but they probably changed to concentric during their passage through the cone scale. Cases of this sort are of course quite frequent.

As to the parichnos strands, Maslen found that in *Lepidostrobus Oldhamius* they began their course in the cone axis as a single strand, just as in the foliage leaf according to Bertrand (1891), and that in the cone the strand remained single in the narrow stalk of the sporophyll; he was of the opinion that the strand disappeared distally. It is of interest in this connection to recall that Scott in his classic work on *Lepidocarpon Lomaxi* (1901, Pl. 41, fig. 10) figures a cone scale with two "lateral gaps, perhaps representing the parichnos". He also refers to a single strand of large-celled tissue occurring in the basal part of the cone scale, which he compares with the single parichnos of the cortical region of a leaf base. An examination of our sections of *Lepidostrobus* shows that in the two best preserved examples the distal part of the scales presents a pair of lateral cavities (Pl. III, fig. 7), so that it becomes entirely probable that in the species represented in our material the parichnos strand, while single in the narrow stalk of the sporophyll, forked and spread out in the wide

distal part of the scale. The interpretation here suggested is strengthened by the account given by Hill (1906) of a pair of parichnos strands in the sporophylls of *Isoetes*. Hill regards the mature condition of the parichnos of recent plants as a mucilage cavity, produced by the breaking down of the tissue which earlier filled the cavity. In recent plants moreover the number of such cavities varies from one in *Lycopodium* to two in *Isoetes*. The evidence at hand seems to indicate not only that the parichnos strands forked in the cone scale of *Lepidostrobus* much as they did in the vegetative leaf, but that in the expanded distal region of the scales the parichnos cavities extended well toward the apex, becoming flattened out in accordance with the shape of the scale, instead of dying out as they did in the leaves.

The so-called transfusion tissue which accompanies the vascular bundles of leaves and cone scales of many if not all lepidodendroids is particularly well represented in our "unknown". It is also figured by Maslen (Pl. 37, fig. 16) in the distal region of the cone scale, and is present in the cone scales represented in our Pl. III, fig. 7. In all the instances of transfusion tissue in lepidodendroids which have come under my observation, the pattern of the tracheids making up this tissue is the same as that of the xylem elements, but the transfusion tracheids are readily distinguished by their greater width and delicacy as well as by their position. We owe the word "transfusion" to von Mohl (1871), although when Renault (1889) found tracheids surrounding the vascular bundle of the leaf of *L. rhodumense*, he styled them "aquéfère". Renault's view (1896) of the function of the layer in serving to store water during periods of drought is somewhat at variance with the claim of Worsdell (1897) that in the gymnosperms transfusion tissue must be considered a rudimentary conducting system. Two more recent writers (Carter, 1911; Takeda, 1913) have considered transfusion tissue to be a water storage region, having in mind the conditions in various gymnosperms. Referring now to the case of the lepidodendroid cone scale, it appears to the present writer entirely natural that the slender strand of tracheids lying adaxial to the leaf-trace should in the wider distal part of the scale spread out and increase in bulk, constituting an efficient storage reservoir serving to keep moist the all-important sporangia. In view of all the evidence, it may be suggested that "transfusion" is a truly misleading term.

Structurally the transfusion layer in lepidodendroids confirms Worsdell's contention as to the morphological nature of the layer in

gymnosperms, viz. that it is an extension of the centripetal xylem, for the general pattern of thickening is in all cases similar to that of the xylem of the bundle, namely, reticulate in lepidodendroids and pitted in conifers. Worsdell's theory has received striking confirmation in the studies of the leaf bundles of *Cordaites principalis*, undertaken by Miss Stopes (1903) and later by Jeffrey (1917) who calls attention to the "intimate connection between the centripetal or cryptogamic wood and the transfusion tissue", which in this species is developed as a double sheath. Turning now to the lepidodendroids, the usual situation appears to be that the xylem of the leaf bundle is separated from the transfusion elements by a parenchymatous (phloem?) layer. It would be satisfying to find in some region an actual contact between these two tracheidal layers, and accordingly a search has been made in the basal region of cone scales and in the cortex of cones of *Lepidodendron*, where the bundle is collateral. In the outer cortex of a cone (*Lepidostrobus*) a typical vascular bundle appears to be mesarch, with a small mass of phloem lying abaxial and a distinct curved area of larger, thick-walled cells (transfusion?) almost surrounding the bundle, and in contact with the xylem on the flanks and adaxial region of the bundle. On account of the condition of preservation of this material, the evidence may be regarded as suggestive rather than demonstrative. Upon the evidence available, the writer is inclined to regard the mantle of reticulate tracheids in leaf and cone scale of lepidodendroids as a real transfusion, or preferably "aquiferous", layer.

On the whole, the evidence submitted appears to warrant the conclusion that the flat organs (Pl. III, figs. 1-6) represent the distal region of cone scales of some species of *Lepidostrobus*, although of a sort not earlier described. It appears moreover that in well-preserved material of *Lepidostrobus* the essential features of structure of the cone scales correspond with those of the leaves of *Lepidodendron* and *Sigillaria* much more fully than earlier accounts lead one to expect, making allowance for the specialised requirements of the cone. Further, it has been impressed upon the writer that we may here be dealing with *Sigillaria* rather than *Lepidostrobus*. As Scott (1920, p. 212) remarks: "The difficulty, in fact, is rather to find constant distinctions between the two genera, than to prove their relationship." The possibility of the scales belonging to *Lepidocarpon* is not denied.

It is a pleasure to acknowledge the courtesy of Dr Seward, who has been kind enough to examine some of the photographs representing the objects in question.

SUMMARY

1. Sections of certain scale-like organs from the Lower Coal Measures are regarded as representing the expanded distal region of the cone scales of an unfamiliar species of *Lepidostrobus*.
2. A comparison between these organs and the leaves and sporophylls of lepidodendroids already described indicates a closer correspondence in structure between cone scales and foliage leaves than the earlier accounts were able to show.
3. The relation of these observations to current theories relating to transfusion tissue is discussed.

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EXPLANATION OF PLATE III

Figs. 1-5 refer to the foliar organs which are regarded as scales of a new species of *Lepidostrobus*.

Fig. 1. Transverse section of half a scale. At the left appears one of the lateral cavities. $\times 16$.

Fig. 2. Central portion of another scale. The lateral cavities are shown, also the central bundle with the U-shaped mass. $\times 32$.

Fig. 3. Longitudinal section through a scale. The peculiar cuticular processes of the epidermal cells are visible on both surfaces of the scale. $\times 70$.

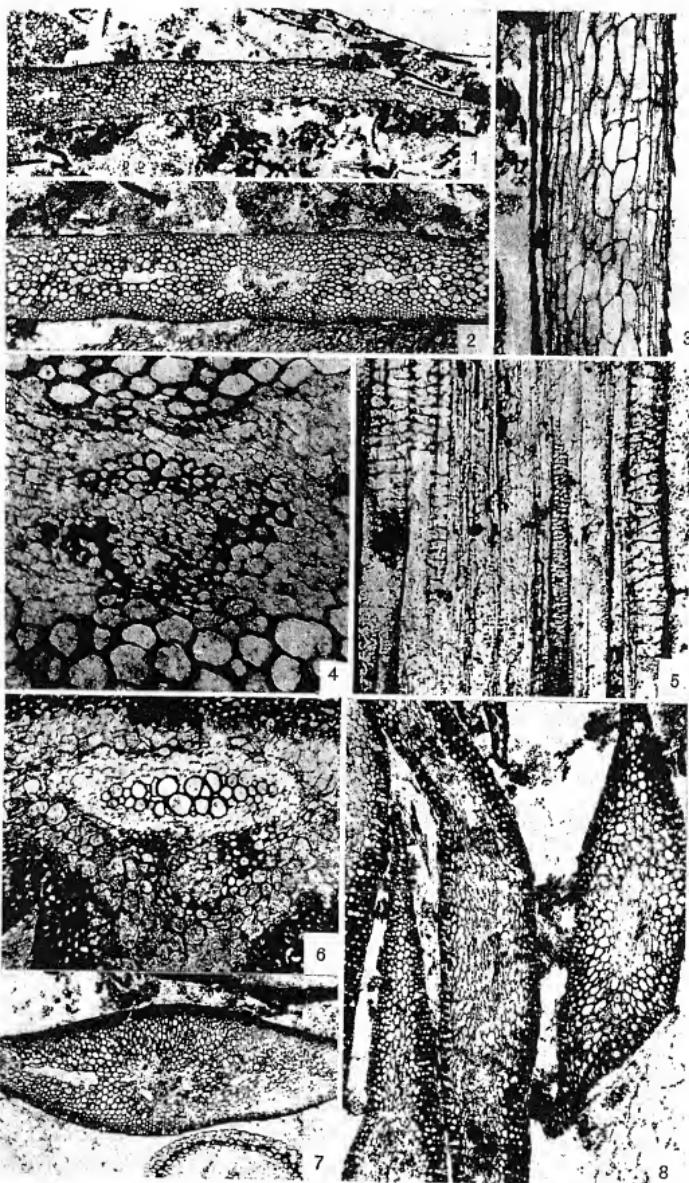
Fig. 4. Transverse section of vascular bundle. In the centre appears the xylem, surrounded by thin-walled cells (phloem?). Abaxial to this is the U-shaped mass enclosing thin-walled cells. Around the whole is the transfusion layer, mostly disorganised. $\times 175$.

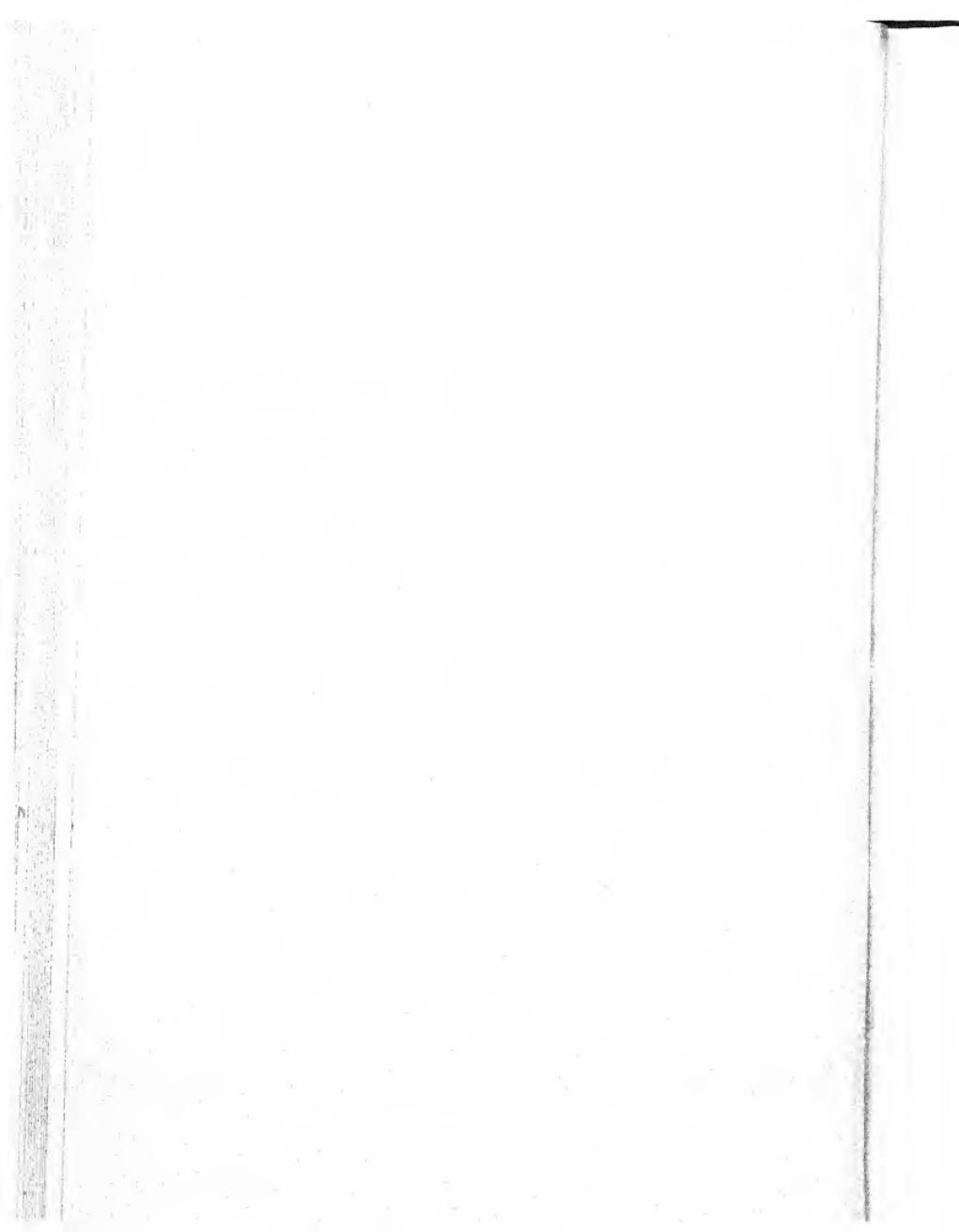
Fig. 5. Longitudinal section through the vascular bundle of the leaf shown in fig. 3. At the centre is the xylem of the bundle. The contrast between its markings and those of the transfusion tracheids is shown. $\times 225$.

Fig. 6. Central region of a leaf of *Sigillaria* sp., introduced for comparison. The diarch xylem is surrounded by phloem, a U-shaped mass lies abaxial to this, and the transfusion sheath surrounds the whole. $\times 75$.

Fig. 7. Transverse section of distal part of a cone scale of *Lepidostrobus*. The lateral cavities are plain, but the vascular bundle is poorly preserved. $\times 20$.

Fig. 8. Cone-scales of another specimen of *Lepidostrobus*. In the middle scale the lateral cavities are partly filled with a disorganised mass of cells (parichnos strands). $\times 50$.

CHRYSLER—ON CONE-SCALES OF *LEPIDOSTROBUS*



REVIEWS

Pollen Grains: their structure, identification and significance in Science and Medicine. By R. P. WODEHOUSE. Pp. 574. McGraw Hill. 1935. \$6.00.

Careful investigation of the structure and comparative morphology of pollen grains has been greatly stimulated in the last twenty years by the development of pollen analysis, and by scientific investigations of hay-fever. It is as a specialist in the second field that Dr Wodehouse has developed his extremely wide and valuable knowledge of pollen morphology, but he has also worked on fossil material and is in touch with the literature here also. To both subjects his new book will be invaluable: partly because of the wide range of pollen grains described and drawn, but much more because it establishes a new standard of thoroughness in microscopic examination of pollen. The illustrated keys to pollen types now consulted by pollen analysts are often quite inaccurate, and their figures reflect a very small fraction of the care and skill which have gone to the making of the illustrations given here.

Apart from a short section on the fossil Gymnosperms, the figures and abundant descriptions are concerned essentially with living pollen and it would, we feel, be a great gain to a future edition of the book, if at least the commoner tree pollens could also be shown as they appear in a subfossil state. They are often more readily recognisable in this condition than fresh. The excellent photographs by the Dutch worker Wassink would demonstrate this point admirably.

The technique of pollen analysis methods is given in a short chapter by Dr Erdman of Stockholm, but it is over-brief, and is largely concerned with advancing the possible merits of the new methods of treating peat samples so as to remove lignin by chlorination, and cellulose by acid hydrolysis. These methods are certainly in some cases very advantageous, but they are in a more experimental stage than a reader might guess, and less than justice has been done to the old method of alkali preparation.

In the course of his work on hay-fever, Dr Wodehouse made daily counts of atmospheric pollen caught on the laboratory roof in Yonkers, N.Y.; he describes the methods employed and gives an extremely interesting figure showing the seasonal variation of the chief pollen types. It would be most interesting, and most valuable to both botanical and medical science to have similar data for different parts of this country.

To the botanist uninterested in the technique of pollen analysis or the investigation of hay-fever, the interest of the book will centre in the chapter on pollen-grain characters, in which Dr Wodehouse explains with care and detail the fundamentals of pollen-grain morphology, and puts forward his own ideas as to the origin of the highly characteristic form and arrangement of pores, furrows and markings which distinguish different types of grain. These characters chiefly concern the exine which is not only protective, but must (by germ pores) be able to allow emergence of the pollen tube and (by special furrows) be able also to accommodate volume changes as the grain takes up or gives off moisture. The germ pore is generally within a furrow, and the two structures may show all degrees of relative importance. The number and arrangement of the furrows are directly traced to the arrangement of the pollen grains during tetrad formation, and the way in which partition takes place. The wall sculpturing is usually disposed symmetrically with respect to the pores. These facts explain the typically triradiate marking and tricolpate

form of the pollen of dicotyledons, in which the pollen grains form in a tetrahedron and separate from one another by furrowing. Abnormal tetrad arrangements are associated with other types of marking and furrow disposition and number. Careful analysis shows that they may all be interpreted mechanistically in terms of stresses set up in the grains during their formation. This background of careful analysis is of great value to the author in his comments on the significance of pollen morphology to phylogeny and gives added weight to the comments on angiosperm and gymnosperm taxonomy which accompany the descriptions throughout the book.

This book will prove essential to botanical libraries: may we hope to have similar treatment extended soon to other species than those of North America.

H. GODWIN.

Laboratory Plant Physiology. By B. S. MEYER and D. B. ANDERSON.
11x8 ins. Pp. vi+107 with 19 figures. Edwards Bros., Ann Arbor. 1935. \$1.75.

In this manual the authors have published a set of laboratory schedules which are in actual use not only in their own departments in the Universities of Ohio and North Carolina, but also in numerous other centres in the United States. They represent a revision of an earlier set issued in 1931, and everywhere bear the hall mark of practical testing and experience.

The ground covered is very wide and includes experiments on solutions, colloidal systems, diffusion, osmosis, permeability, water relations of all kinds, assimilation, metabolism, translocation and respiration. There are also useful general directions on such matters as simple glass working, the use of balances, germinating seeds and the preparation of special reagents. No experiments on growth and irritability are included.

The experiments are intentionally of very different degrees of complexity, some being old favourites of the elementary class, while others would tax the ability and patience of advanced students as far as these can reasonably be expected to go. By judicious choosing a highly instructive course could be selected from these schedules for a class of beginners, and they would certainly be well worth careful study by anyone about to undertake practical instruction in a school. No expensive special apparatus would be required. At the other extreme are experiments involving quantitative determinations amply elaborated for use in degree courses. With such diverse material it is evident that a good deal of separation would be necessary to obtain the best results and the authors themselves recommend it.

W. O. JAMES.

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WATER RELATIONS AND OSMOTIC PRESSURES OF PLANT CELLS

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(With 3 figures in the text)

INTRODUCTION

THE work done by de Vries, extended in various directions by subsequent workers, Dixon and Atkins, Ursprung and Blum, and many others, has given a picture of the water relations of the plant cell which is accepted almost universally at the present day. This universal view may be concisely stated; one assumes that the gross pressure sending water into a cell is the osmotic pressure of the vacuole contents. This is opposed by certain pressures acting in the opposite direction, the osmotic pressure of the external solution and the pressure due to the stretched cell wall (wall pressure). The resultant of all these pressures is the net pressure sending water into the cell which is commonly termed the suction pressure.

Similarly when a cell is immersed in a solution which brings about the condition of incipient plasmolysis, it is assumed that the osmotic pressures of the internal and external solutions are equal, that the living protoplast acts as a semipermeable membrane, and that the passage of water through it is a purely physical osmotic phenomenon.

It is rather remarkable that apparently no attempts have been made to obtain experimental evidence in support of the assumption that the living protoplast merely acts as a semipermeable membrane similar to artificial membranes and that no contribution to the passage of water is made by metabolic or other processes bound up with the living nature of the protoplast.

There are various reasons for doubting this passivity of the living protoplast. It is, for example, well known that the passage of mineral salts through the protoplasts of cells from potential growing tissue

does not follow the course that the laws of diffusion and osmosis would lead one to expect (Hoagland & Davis^(5, 6), Steward⁽⁹⁾). Entry of salts into the vacuole takes place against the diffusion gradient, and this process is associated with the respiratory activities of the tissue. Active water secretion is well known in both plant and animal kingdoms though the mechanism bringing it about is unknown (cf. also⁽³⁾).

It consequently seems to us quite unjustified to assume without proof that the protoplast of the plant cell has no water-secreting power comparable with its power of secreting salts into the vacuole.

The gross pressure sending water into the cell can be obtained by plasmolytic methods. The osmotic pressure of the vacuole contents can be obtained by determination of the freezing-point of the expressed sap. These two should be equal if the protoplast is really a passive semipermeable membrane. Actually we find that in certain tissues the gross water absorption pressure (which we will term A) does equal the osmotic pressure of the internal solution (P_i), but in many other tissues A is much greater than P_i . In these latter tissues therefore the protoplast would appear to be actively secreting water from the external medium into the vacuole.

EXPERIMENTAL METHODS

(a) The osmotic pressure of the cell sap was determined by the cryoscopic method. Cell-sap samples were obtained by Dixon's method⁽⁸⁾; the tissue was frozen and killed by immersion in liquid air and on thawing the sap was pressed out. Samples were kept at about -10°C . until their freezing-points were determined. The freezing-points were determined by a special Anschütz type thermometer having a very small bulb; 1 c.c. samples of sap were used. The freezing-points were determined to the nearest 0.02°C . It may be pointed out that one obtains by this method the average osmotic pressure of the cell sap of a mass of tissue, and it may be assumed that this method of obtaining the sap provides a sample not much different in composition from that present in the living cell.

(b) Some discussion is desirable of the plasmolytic methods for the determination of the osmotic value (or, as we shall call it, the water-absorption pressure) of the cell. The standard value which is usually determined is the limiting plasmolysis value (grenzplasmolysewert) which is the osmotic pressure of the solution in which half the cells show plasmolysis and half are unplasmolysed. Previous investigators have not explained exactly how they have arrived at this value, and in many cases it is not at all clear whether the con-

dition of "half-plasmolysed" has been found by careful and accurate counting or whether it is a rough visual impression obtained by a cursory examination of the tissue.

It appears to us that the correct procedure should be as follows: tissue is placed in a series of solutions of the plasmolyticum of various osmotic pressures. After a given time interval the percentage of the cells in the plasmolysed state in each solution is determined. Obviously the larger the number of cells counted the better will these results be. In our procedure the cells in a given field of view are counted, a new field is then taken, and so on until 100 cells have been counted. The results are then expressed graphically as in Fig. 1,

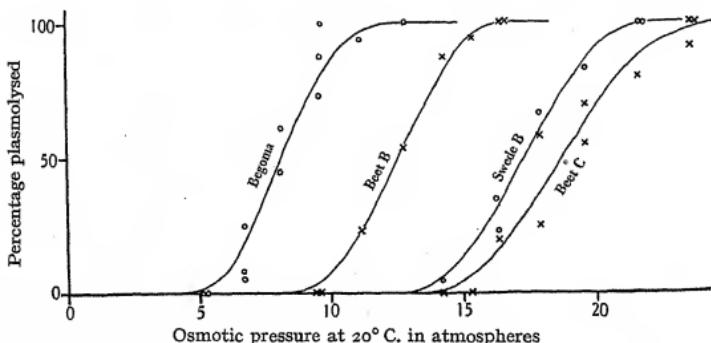


Fig. 1. Percentage of cells of beet (—+—), swede (—+—), and *Begonia* (—○—) plasmolysed in sucrose solutions of various osmotic pressures.

where the percentages of the total number of cells in the plasmolysed state are given as ordinates and the osmotic pressures of the plasmolyticum as abscissae;

The experimentally determined points fall on or about a typical S-shaped curve, and the point of inflexion of this curve evidently gives the limiting plasmolysis value.

From the point of view of this particular research, what we wish to obtain is the mean osmotic value of cells which are in the same condition of turgor as those used as a source of sap for cryoscopic determination. The treatment of this problem accepted at present is expressed in the equation given below in which Hofler's notation is used:

$$P_e \cdot V_e = P_t \cdot V_t \quad \text{and} \quad P_e = P_t \cdot \frac{V_t}{V_e},$$

where P_c and V_c are the osmotic pressure and volume of the cell contents of the turgid cell, and P_t and V_t those of the cell at limiting plasmolysis. P_t is taken to be equal to the osmotic pressure of the solution which brings about the condition of limiting plasmolysis in Hofler's treatment. This latter assumption, that at limiting plasmolysis the osmotic pressure of the cell contents is equal to that of the external solution, is not correct in many tissues as we shall show in the description of the experimental results, and the treatment above therefore requires modification.

As we shall show the gross water-absorption pressure of the turgid cell is greater than the osmotic pressure of its cell contents, so we may write

$$A_t = P_t + X,$$

where A_t and P_t are the water-absorption pressure of the cell and osmotic pressure of the sap, respectively, when the cell is turgid, and X is an unknown additional pressure sending water into the cell; this may be an electrical endosmotic pressure or it may be generated by some activity of the living protoplast as yet unthought of. Similarly when the cell is in the condition of limiting plasmolysis, we have the relationship

$$A_l = P_l + X.$$

Now A_l is equal to the osmotic pressure of the solution which brings about limiting plasmolysis; the value of P_l is known from cryoscopic determinations, and it is the object of this research to compare A_l with P_t and so evaluate X . It should perhaps be pointed out that the values of A_l and A_t are nearly equal, as are also the values P_t and P_l . We know further, from the gas laws, that

$$P_t \cdot V_t = P_l \cdot V_l,$$

where V_t and V_l are the volumes of the vacuole of the cell in the states of turgor and limiting plasmolysis, respectively. Combining these three equations we obtain the following:

$$A_t = A_l \cdot \frac{V_l}{V_t} + X \left(1 - \frac{V_l}{V_t} \right)$$

and

$$A_l - P_t \cdot \frac{V_t}{V_l} = X.$$

The determination of P_t involves the use of a large number of cells and that of A_l of a number of separate single cells. The values of A_l for individual cells of certain fairly uniform tissues fall on frequency distribution curves given in Figs. 1 and 2. To apply the above equation we are using the experimentally determined modal value of A_l ,

and taking the mean osmotic pressure of the expressed sap as equivalent to the modal osmotic pressure of P_t , the individual cells of the tissue.

This is entirely justified if there is no correlation between cell size and the osmotic pressure of the cell sap. On the other hand, clearly if, say, the large cells have more dilute sap than the small cells, the expressed sap will have a lower osmotic pressure than the modal osmotic pressure. There is no evidence for any such correlation in any of the tissues examined by us, nor so far as we can see is it possible to obtain evidence. It is, however, possible to show that there is no correlation between the value of A_t and the size of the cell.

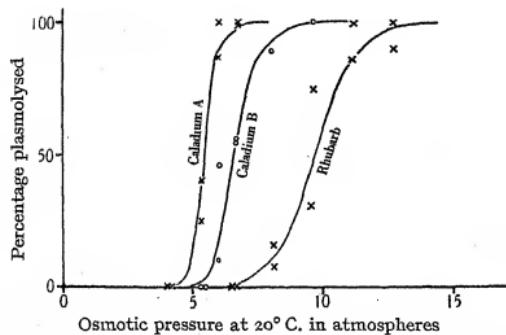


Fig. 2. Percentage of cells of rhubarb petiole (—+—) and *Caladium* petioles (A, —+—; B, —○—) plasmolysed in sucrose solutions of various osmotic pressures.

True limiting plasmolysis cannot be detected, and it is quite clear that at the first detectable stage of plasmolysis the volume of the vacuole must be smaller than at true limiting plasmolysis. Any error due to this is however allowed for in the factor V_t/V_i in the equation above.

There remain to be considered certain criticisms of the plasmolytic method advanced by Ernest(4). She rightly points out that the assumption that the cell contents and external solution are necessarily in equilibrium after about 30 min. is unjustified, and she adds that the rate of passage of water through the protoplast will be proportional to the osmotic pressure difference across it. Thus when the internal and external pressures are nearly equal the rate will be very small and it is implied by Ernest that equilibrium will therefore be attained very slowly. It does not, however, follow that the attain-

ment of equilibrium will be much delayed under these conditions, since the actual volume of water which must be transferred is also correspondingly very small. Ernest's results appear to indicate that equilibrium is closely approached after about 60–100 min. immersion in the case of the tissues examined by her. These results are expressed graphically, concentrations of sucrose required for "inception of plasmolysis" are given as ordinates and times of exposure as abscissae; exactly what the former term is, is not explained, nor can one judge at all of the statistical significance of the results.

Typical results of our own on this matter are cited in Table I.

TABLE I. Percentage of *Rhoeo* epidermis cells plasmolysed after various durations of exposure to sucrose solutions of various concentrations

Molarity of sucrose	Time of exposure (min.)				
	5	15	20	40	80
1·0	90	100	100	100	100
0·45	8	80	100	100	100
0·40	0	88	98	100	100
0·30	0	67	50	66	60
0·25	0	19	30	38	40
0·20	0	—	0	0	0

The variability of the results in the fourth line is ascribable to sampling errors. The results of this and a number of other experiments indicate that equilibrium almost certainly is attained after about 30 min. exposure in the case of the various tissues examined by us. In all cases, however, tissues were examined after both 30 and 60 min. exposure.

(c) Whenever the limiting plasmolysis method is used it is a matter of the first importance, as de Vries pointed out, that it should be possible to detect the minutest trace of plasmolysis, and the coloured epidermis of *Rhoeo discolor* is, of course, the classic object of such researches. In general, only tissues of which the cells have coloured cell sap are suitable; in this particular research it is also essential that a fair bulk of uniform tissue should be obtainable for cryoscopic determinations.

Of a large number of plants used by us, greatest reliance is to be placed on results obtained from the following: beet (*Beta vulgaris*, garden varieties) root, *Caladium bicolor* petiole, *Rheum* sp. (garden variety known as "strawberry rhubarb") petiole, *Begonia rex* petiole, all of which have coloured cell saps. It is fairly easy to detect plasmolysis in the ground tissue cells of the root of the swede, and the results using this tissue are also fairly reliable. With a number of

other tissues used the results are definitely less reliable owing to the difficulty of detecting the first traces of plasmolysis.

For the plasmolytic researches sections of tissue were injected with plasmolyticum by a number of successive evacuations. Ernest objects to the use of sections because of supposed pathological effects produced by the cell sap of ruptured cells. Our results show, we believe, that some of these supposed pathological actions of juice are to be expected provided the cell sap does *not* injure the protoplast when applied outside the cell. But in any case our treatment of the sections ensures that their own cell sap is very quickly replaced from the intercellular spaces by plasmolyticum.

EXPERIMENTAL RESULTS

(a) *Beet.* Cylindrical samples 6 mm. in diameter and 25 mm. long were cut parallel to the long axis all at the same distance from the centre. Very similar samples were thus obtained. Sections from the top and bottom of the cylinders were taken for use in plasmolytic measurements; the cylinders were then killed in liquid air, and, on thawing, the sap from each was pressed separately and the freezing-point of each sap sample was determined. Three different individual beetroots were used, each of a different variety; these three varieties differed markedly in osmotic pressure and sugar content. We have unfortunately no certain information of the names of these varieties and they will be referred to here as A, B, and C.

The depressions of freezing-point (Δ) of the samples of sap from each of these beets are given in the second column of Table II, and the corresponding osmotic pressures are given in the third column, expressed in atmospheres at 20°C .

The plasmolytic results are given in detail in Fig. 1 for beets B and C. Each point on the curve refers to a count of 100 cells. It will be noted that the point of inflexion falls (within the limits of sampling error) at the 50 per cent plasmolysis point, which therefore gives the modal limiting plasmolysis value for the cells of the tissue. This is the term A_1 of our previous discussion; the values of this are recorded in the third column of Table II.

It is also necessary to obtain the value of V_t/V_i , the ratio of volumes of turgid and incipiently plasmolysed vacuole. It is only possible to get a rather rough estimate of this. The ratio of volumes of turgid/flaccid cell, which is termed by Hofler the degree of turgor stretching, can be obtained by measuring the linear dimensions of a thin slice of tissue before and after plasmolysis. In the case of the

three beets used the shrinkage was found to be between 2 and 4 per cent. When plasmolysis is just detectable the volume of the vacuole is somewhat smaller than its volume at true limiting plasmolysis. To evaluate the ratio of the volumes, we assume that the volume at true limiting plasmolysis is proportional to the (area of field of the cell) $\times \sqrt{\text{this area}}$ and that volume of the vacuole when the first trace of plasmolysis is visible is proportional to (area of field of vacuole) $\times \sqrt{\text{this area}}$.

In our experience it is possible to detect plasmolysis when the shrinkage of the vacuole is as little as 1 per cent of the volume it occupies at true limiting plasmolysis, except in the case of very small cells or cells which have indistinct vacuoles.

We can state with some confidence that the value of V_t/V_i in the beetroots examined is between 1.00 and 1.05.

(b) *Swede*. Samples were taken in exactly the same way as has been described for the beetroot. Freezing-point depressions of the expressed sap are given in Table II and plasmolytic data for swede B in Fig. 1. Two different swede roots were used distinguished as A and B; they were, however, both of the same variety. The value of V_t/V_i was again found to be roughly 1.05.

(c) *Begonia Rex*. Petioles of approximately the same age were taken and 25 mm. lengths were frozen in liquid air and the sap of each expressed and the freezing-point depression determined. Before freezing, sections were cut transversely off each end for immersion in the plasmolytica. The experimental results are given in Table II and Fig. 1. Material was collected on two separate occasions and the two batches of material are distinguished as A and B. The two batches of material were essentially similar and were cut from the same plant.

(d) *Rheum*. Petioles of the "strawberry rhubarb" were used in the same manner as the begonia petioles. This variety of rhubarb has anthocyan pigment in the ground tissue cells as well as in the epidermis. The sections used for plasmolysis were cut longitudinally in the case of the rhubarb petiole both on account of the markedly slight deformation of the cell caused by cutting a longitudinal section and because traces of plasmolysis are more readily seen in longitudinal section in rhubarb. Results are given in Table II and Fig. 2.

(e) *Caladium bicolor*. Petioles were used in the same way as the rhubarb petioles. Two batches of material were used, both collected from the same plant but on different occasions. Batch A was slightly younger than B. Results are given in Table II and Fig. 2.

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TABLE II. Summary of osmotic pressure and plasmolytic data.
Osmotic pressures are expressed in all cases as atmospheres at 20° C.

	Freezing-point depression °C.	Osmotic pressure of expressed sap $(=P_t)$	External osmotic pressure at limiting plasmolysis $(=A_t)$	X $(=A_t - P_t \cdot \frac{V_t}{V_l})$
Beet A	1.43	15.5	23.4	7.1
Beet B	0.67 0.685 0.77 0.83 0.775 0.70			
Beet C	Av. 0.74 0.96 0.84 0.92 1.01	9.5	12.6	2.6
Swede A	0.88	11.3	17.8	5.9
Swede B	0.86 0.99 0.95 0.96 0.89 0.89 0.88			
Begonia A	0.41	5.3	8.0	2.4
Begonia B	0.45 0.40 0.45			
Rhubarb A	0.58	7.5	8.1	+0.2
Rhubarb B	0.73 0.68 0.61 0.66			
Caladium A	0.67	8.6	9.5	+0.5
Caladium B	0.45 0.45 0.44 0.47 0.45 0.45 0.46 0.44	5.8	5.5	-0.5
	Av. 0.45	5.8	6.4	+0.4

The sample A in each case above consisted of sap from about 10 g. of material in the form of six similar cylindrical pieces about 25 × 6 mm.

TABLE III
Plasmometric data relative to *Caladium B* in 0·45 m. sucrose

Initial length	Initial breadth	Radius of spherical plasmolysed protoplast	Initial osmotic pressure
26	44	18	7·2
30	30	15	7·6
30	24	12	6·0
32	24	11	5·3
38	38	19	7·6
25	22	11	6·8
			Av. 6·75

The cells of the ground tissue of the *Caladium* petiole are so regular that it is possible to apply the plasmometric method to determine the "osmotic value" of individual cells with fair accuracy. Hofler's expression cannot be used as the cells are hexagonal, not circular, in section. These hexagons are not quite regular, so the determination as applied to an individual cell may be inexact, but the mean of many determinations is probably fairly reliable. The volume of a regular hexagonal cell is $0\cdot87d^2l$, where l is the length and d the distance from the middle of one side to the middle of the opposite side. It is important not to measure the distance between opposite corners. The experimental results given in Table III show quite good agreement between plasmometric and plasmolytic methods considering the limitations of both methods. *Caladium B* was used.

DISCUSSION

Expressions have been developed in our discussion of the accuracy of plasmolytic methods involving (for the case of a single cell) the following terms: A_l , the gross water absorption pressure of the cell at limiting plasmolysis which, if equilibrium is established, must equal the osmotic pressure of the external solution; P_t , the actual osmotic pressure of the cell contents of the turgid cell; X , a pressure, additional to the osmotic pressure of the cell contents, sending water into the vacuole. (Hitherto it has been assumed that there is no such pressure operating, i.e. that $X=0$.)

We have shown that

$$X = A_l - P_t \cdot \frac{V_t}{V_l}$$

The legitimacy of using the modal values of the limiting plasmolysis value and the mean osmotic pressure of the expressed sap as A_l and P_t respectively has been discussed.

Using these values for A_t and P_t we obtain the values of X , which are recorded in the last column of Table II. In calculating these values of X , V_d/V_t has been taken as 1.05, which for these tissues examined is probably a maximum value. Minimum values for X are consequently obtained.

This additional pressure, X , which we propose to term the secretion pressure, has values ranging from about 3 to 7 atmospheres in the roots of beet and swede, 2 atmospheres in *Begonia* petiole, and values which probably do not differ significantly from zero in the petioles of rhubarb and *Caladium*. It is perhaps significant that the tissues showing this secretion pressure are potential growing tissues and comparison may be instituted between these results and those of Berry & Steward (2), who show that bromide absorption is characteristic only of potential growing tissue.

Statistical evaluation of the significance of the values of X , the secretion pressure, has not been carried out, since the significance really depends on the validity of the assumption that the osmotic pressure of the mixed sap from all the cells of a tissue has the same value as the modal cell sap osmotic pressure. No mathematical test of the validity of this assumption is obtainable, although we believe it to be reasonable.

There is, however, an important line of evidence substantiating our conclusions that there is a secretion pressure of quite considerable magnitude in certain tissues, and that it is close to zero in others. Consider the data relating to beetroot B in Fig. 1. The frequency distribution of the osmotic values (A_t) for the cells are given by the plasmolysis-osmotic pressure curve; the greatest or modal frequency is at 12.6 atmospheres and the frequency decreases towards zero, so that no cells are found having osmotic values lower than 9.5 or higher than 16.0. The modal cell osmotic pressure, which we may take as equal to that of the expressed sap, is in this case found to be 9.5 atmospheres. The probable course of the frequency distribution curves of osmotic pressure of cell sap of individual cells and of water absorption pressure (as determined plasmolytically) are given diagrammatically in Fig. 3.

It is quite clear that no cells should be plasmolysed when tissue is mounted in its own expressed sap, since the modal osmotic pressure falls outside the "tail" of the frequency distribution curve of water absorption pressures. Actually we found experimentally that no cells were plasmolysed.

A sample of this beetroot sap was concentrated (1.00 c.c. was

allowed to lose 0.27 g. of water, the osmotic pressure was thus raised from 9.5 to 13.1 atmospheres) and sections from the same piece of beetroot were injected with this. Partial plasmolysis resulted. The percentages plasmolysed were found to be 32, 42, and 60 per cent in separate counts of 100 cells each. The extent of plasmolysis is thus approximately the same as that induced by a sucrose solution of the same osmotic pressure, 13.1 atmospheres.

Applying the same reasoning to the case of *Caladium bicolor*, we obtain frequency distribution curves of the water absorption pressure and osmotic pressure of the cell sap which are also given diagrammatically in Fig. 3. Here the modal sap osmotic pressure equals the modal water absorption pressure and the two curves may be expected

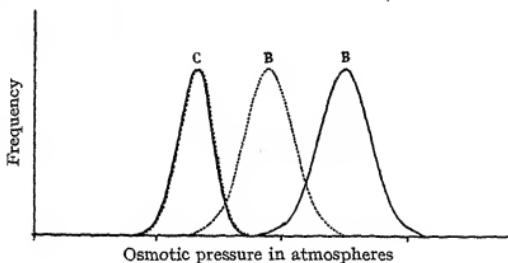


Fig. 3. Frequency distribution of osmotic pressures of cell saps (---) and water absorption pressures of cells (—) in the cases of beet B and *Caladium* C. Beet curves are labelled B and *Caladium* C.

to coincide. Consequently the modal, or expressed, sap should be expected to cause plasmolysis of the half of the cells whose water absorption pressures are lower than the modal water absorption pressure.

Sections were injected with expressed sap from the same piece of petiole and mounted in this sap, and it was in fact found that marked plasmolysis took place. The results of a number of separate counts of 100 cells gave the following percentages plasmolysed: 51, 41, 44 per cent. The agreement with our expectations is thus closely followed.

Similar results to these have been obtained by other workers though this interpretation has not been advanced. Dixon (3) has found tissues whose cells are not plasmolysed by their own expressed sap. Previous investigators have not emphasized the remarkable character of this result, which can only mean that the modal osmotic pressure of the cell sap falls outside the "tail" of the frequency dis-

tribution curve of the water-absorption pressures and that there must therefore be active water secretion into the vacuole in addition to the flow due to an osmotic pressure gradient.

Tissues are also known in which plasmolysis is brought about by the juice exuding from the cut cells composing part of the section examined. Hitherto this has been ascribed to the injurious effects of the cell sap on the outsides of the protoplasts (Ernest⁽⁴⁾). In our view this plasmolysis, which we have never found to exceed 50 per cent, is to be expected on statistical grounds if the water secretion pressure into the vacuole is zero or very low. Furthermore, it seems to us quite unreasonable to suppose that cell sap which does not injure the inside of the protoplast should exercise such a rapid and peculiar injurious effect on the outside.

Diluted *Caladium* sap does not plasmolyse cells from the same piece of tissue as we should expect on our view. If its action were to cause injury, dilution to 75 per cent of its original strength would hardly be expected to remove these supposed toxic properties completely, yet this dilution was found to completely prevent the plasmolysis caused by it.

The actual determined values of X , the water secretion pressure, given in Table II, are not claimed by us to possess a very high order of accuracy, but we submit that these values taken in conjunction with the findings and discussion regarding the effects of immersion of tissue in its own juice establish with some certainty the contention that considerable water-secretion pressures exist in addition to the osmotic flow of water.

Our results are too few to generalize with much confidence, but it appears that these high values of the water-secretion pressure are found chiefly in potential growing tissues.

It is unprofitable to speculate in detail on the mechanism of this water secretion. Osterhout⁽⁷⁾ has found that the inside of the cell of *Nitella* is negatively charged relatively to the outside, and if as is likely the protoplast carries a negative charge there would be electrical endosmotic flow of water into the vacuole from the external medium. One would expect that the magnitude of electrical endosmotic water secretion would be affected if electrolytes were used as plasmolytica instead of sucrose. This point is being investigated, as is the question of whether the secretion pressure is affected by temperature or respiration rate.

Finally one must allude to the fact that plasmolytic determinations of permeability are complicated if not actually invalidated by

failure to recognize the existence of this active water secretion. Where a leaf tissue such as *Rhoeo discolor* is used (Bärlund⁽¹⁾) probably one is justified in assuming the absence of active water secretion, but potential growing tissues such as *Beggiotaea* may show active water secretion into the vacuole. It is well known (cf. Ruhland & Hoffmann⁽⁸⁾) that these particular tissues show certain marked differences in behaviour from *Rhoeo* so far as "permeability coefficients" are concerned.

SUMMARY

1. It is pointed out that no attempt has been made to verify the assumption that the protoplast of the plant cell acts merely as a passive semipermeable membrane without showing active "secretion" of water. Methods of investigating this matter are discussed.

2. It is shown that the so-called "osmotic value" of the cell sap as determined by the plasmolytic method is markedly greater than the osmotic pressure of the cell sap as determined by the cryoscopic method in certain tissues (roots of beet and swede, petiole of *Begonia*), but in certain other tissues the two may be equal (petioles of *Caladium* and *Rheum*).

3. The former tissues thus show positive water secretion from the external medium into the vacuoles, in addition to the flow inwards caused by the osmotic pressure of the cell sap. In the latter, *Caladium* and *Rheum*, flow of water is conditioned by the osmotic pressures only.

4. It is shown that the cells of tissue possessing active water secreting powers should not be plasmolysed by their own expressed sap, whereas juice from tissues not possessing water secreting powers should plasmolyse approximately half of the cells of the tissue. This is found experimentally, and confirms the conclusion that the osmotic pressure of the vacuole is not the sole force sending water into the cell, at any rate in potential growing tissues.

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ADDENDUM

Since this was written our attention has been called to the paper by Buhmann (*Protoplasma*, **23**, 579, 1935) in which somewhat similar results are quoted. The difference between cryoscopic and plasmolytic values of the cell osmotic pressure is ascribed by her to adhesion of the protoplast to the wall, a view from which we dissent for many reasons amongst which are the following: the magnitude of the difference is too large; deformation of the cell wall such as is demanded by the adhesion view is not observed; it should be impossible to observe cells in equilibrium in the condition of limiting plasmolysis which in actual fact are seen in large numbers.

UPWARD EFFECTS OF AUXIN IN COLEOPTILES AND STEMS

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(With 3 figures in the text)

INTRODUCTION

IN experiments recently carried out at Oxford Miss Le Fanu (1936) has applied a paste of lanoline containing hetero-auxin to the stems of pea seedlings below the elongating region, and has found that the elongation of the parts above was retarded; although the same paste, when applied near the top of the elongating zone, accelerated the elongation of the parts below, in agreement with the results of Thimann & Skoog (1934).¹ This interesting result put the present writer in mind to test the effect of hetero-auxin on the growth of oat coleoptiles, when applied near the base, since these coleoptiles are the original test objects for the effects of the auxins. Experiments of this kind have indeed been performed by Laibach & Kornmann (1933), who attached small agar blocks containing auxin to the intact sides of coleoptiles at various levels. They state (p. 415) that negative curvatures (indicating acceleration of growth) were caused only in the parts below the blocks and never above them. But a different result, following a slightly different method of application, will be reported here.

SHORT-TIME UPWARD EFFECTS IN COLEOPTILES AND SUNFLOWER HYPOCOTYLS

The method used for the present experiments was the following. Oats were soaked and sown in boxes of soil with the top of the seed just projecting above the surface. As soon as the coleoptiles were just visible, the boxes were placed under overhead light for some hours, to repress the mesocotyl, and were then transferred to a dark room. The hetero-auxin paste used was made by stirring up a 1 in

¹ Zimmerman & Wilcoxon (1935, pp. 217, 220) and Fisch nich (1935, p. 554) have also reported retardation of stem growth by hetero-auxin paste, but without making the point that it occurs only when the paste is applied below the growing region.

1000 solution of hetero-auxin in distilled water with an equal part of anhydrous wool-fat (see Laibach, 1933). Operations were performed by red light. When the coleoptiles were 1.5 or 2 cm. long, they were decapitated by Stark's method and the included leaf was pulled out.¹ The coleoptiles were wiped dry, if moist on the surface; a small blob of the hetero-auxin paste was placed on one of the narrow sides of the coleoptiles near the base, and a ring of vaseline was placed just above it round the coleoptile, as a precaution against upward movement of hormone in any possible surface film of moisture. During the experiment the air was kept fairly damp, but not so damp as to cause bleeding: the coleoptiles were decapitated again every 2 hours to prevent physiological regeneration of new tips. The temperature was kept at 19° C.

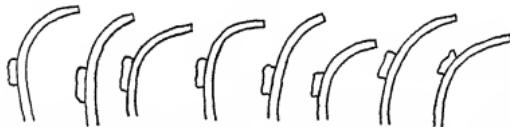


Fig. 1. Coleoptiles with hetero-auxin paste on narrow side, after 5½ hours. $\times \frac{2}{3}$.



Fig. 2. Coleoptiles with hetero-auxin paste on narrow side, after 6½ hours. $\times \frac{2}{3}$.

The illustration (Fig. 1) shows the result of one experiment with eight coleoptiles after 5½ hours: it was made by carefully pinning down the coleoptiles without distortion and running a pencil round their outlines: this rough method suffices for the present purpose. In all the coleoptiles the parts above the paste have curved strongly away from the side to which it was applied, indicating that the hetero-auxin *accelerates* the elongation of the side of the coleoptile above it, just as it does that of the side of the coleoptile below it: for in controls pure lanoline had no effect. The curves, in the parts above the top of the paste only, range from 40° to 92°, the mean being 71°. They reach to the ends of the coleoptiles, which are from 13 to 18 mm. distant, and at an earlier stage, after only 3½ hours, it was noted that in six of the eight coleoptiles the curves had already spread upwards from 12 to 15 mm. above the top of the paste.

¹ The included leaf comes out most easily when the plants are rather damp: forceps with cork at the tips are helpful.

Two other experiments with seven and eight coleoptiles respectively gave similar results. All the coleoptiles curved negatively, the mean curvatures above the paste in the two experiments after 6½ hours being 57° and 35°. The former of these two experiments is illustrated in Fig. 2.

In still another experiment the coleoptiles, five in number, were not decapitated. Yet even these all developed distinct, though less strong, negative curvatures in the parts close above the paste, the mean curvature after 6 hours being 29°. The extreme tips had curved up geotropically in the opposite direction.

From these results it may reasonably be concluded that, contrary to what is generally believed, the auxins can travel in the morphologically upward direction in coleoptiles, and that they can do so more effectively than by simple diffusion. It is further clear that when travelling upwards in coleoptiles they accelerate growth just as they do when travelling downwards; and so also must they do when travelling sideways, since there were also strong negative curvatures within the short zone to which the paste was applied.

Laibach & Kornmann (1933, p. 415) have stated that they never obtained curvatures above their blocks of auxin agar; but one of their own photographs (p. 411, Fig. 11, upper row) shows clearly that, in four of the five coleoptiles illustrated, there actually *were* negative curvatures, in the parts above the blocks, but below the apical regions which had curved up geotropically in the opposite direction. Indeed, one of these curvatures above the blocks is of 25°, although the coleoptiles had not been decapitated. If in their other experiments they obtained little or no curvature above the blocks, the explanation may be that their blocks were so very small, as their photographs show.

The question may be raised whether the upward transport of hetero-auxin takes place in the conducting strands—perhaps in the transpiration stream. This question cannot be answered by placing the plants in a saturated atmosphere, since then they bleed. So two further experiments were performed which were similar to the previous ones except that the hetero-auxin paste was placed on one of the *broad* sides of the coleoptile near the base. The reason for so doing was that the two conducting strands are at the two narrow sides of the coleoptile. Consequently with the paste on one of the broad sides, midway between the two bundles, negative curvatures (if any were to be obtained) could not be ascribed to a difference in the amounts of hetero-auxin transported upwards by the bundles.

Van der Weij (1932, p. 488) has tested by this method the downward transport of auxin in coleoptiles when applied in agar blocks. He states, without giving details, that he obtained slightly greater curvatures when the blocks were placed on top of one of the broad sides. On the other hand, Laibach & Kornmann (1933, p. 409 seq.) regularly obtained much greater curvatures below their blocks of auxin agar when they attached them to one of the narrow sides.

In the present experiments it was found that when the paste was put on a broad side near the base, the negative curves above the paste were much less strong and developed much more slowly. Two experiments were made, with six and seven coleoptiles respectively. After periods of $6\frac{1}{2}$ and 10 hours with repeated decapitations, only about half of the coleoptiles were slightly curved above the paste. But when they had been left overnight (the temperature dropping to $14^{\circ}\text{C}.$), then finally after a total of 22 or 23 hours all were curved negatively above the paste, as illustrated in Fig. 3, the mean curve being, however, only 19.3° . At the top the coleoptiles have curved up the other way by geotropism.

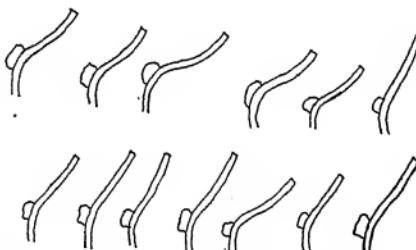


Fig. 3. Coleoptiles with paste on broad side, after 22 or 23 hours. $\times \frac{3}{4}$.

It must be concluded that hetero-auxin can be transported upwards in the parenchyma of coleoptiles, but that it is transported upwards more rapidly and effectively in the conducting strands (not necessarily in the transpiration stream). The results of Laibach & Kornmann (1933, p. 409), already mentioned, show that the same is true for downward transport.

By similar experiments evidence was obtained that in hypocotyls of dark-grown sunflower seedlings also hetero-auxin can be transported upwards and accelerate elongation. The seedlings were decapitated near the top of the hypocotyl when this was about 3 cm. long, and $2\frac{1}{2}$ hours later a blob of hetero-auxin paste (1 in 1000) was

put on one side of the hypocotyl near the base. After 10 hours (at 18° C.) the hypocotyls had curved away in the parts above the top of the hetero-auxin paste. The curves ranged from 10° to 30°, and extended from 8 to 17 mm. above the paste.

Upward transport of hetero-auxin and other growth substances applied in lanoline has been demonstrated by Hitchcock & Zimmerman also (1935, pp. 459 and 467) in tobacco and marigold plants. The growth substances caused epinastic curvatures of leaves and stems above, and some part of the upward transport must sometimes have taken place in the transpiration stream; for the hetero-auxin, when applied in this way, sometimes travelled up through a dead zone, but only with difficulty and only when applied to "soft tissue". But they consider that most of the growth substance, when applied in lanoline, is transported otherwise than in the transpiration stream, since transport is then largely independent of atmospheric conditions (p. 467). Fisch nich also (1935, p. 570) has reported upward transport of hetero-auxin applied in lanoline to *Coleus* plants: it caused epinasty of leaves above. He concludes that in this species the hetero-auxin is transported mainly in the bundles.

The experiments which have led to the general belief that auxin is transported only in the morphologically downward direction in stems and coleoptiles have mostly been performed with isolated lengths cut from these parts. Usually the isolated length of tissue has been placed, either upright or inverted, with one end touching a block of agar containing auxin, and the other end touching a block of pure agar; and after some hours it has been determined by Went's method (1928) how much auxin has been transported into the latter block. The first such experiments were made by Went (1928, pp. 57-8) with oat coleoptiles, and the same method has been used by Van der Weij (1932, p. 446, 1934) also for oat coleoptiles, by van Overbeek (1933, p. 582) for hypocotyls of *Raphanus sativus* and by Dijkman (1934, p. 414) for hypocotyls and "plumules" of *Lupinus albus*. The first three of these workers concluded that transport took place only towards the morphological base, and the last described it as being "polar" in the same sense. Still other workers have reached the same conclusion but have not given results in detail. But it is worth noting that all four of the workers mentioned did obtain some slight curvatures in a sense which indicated a slight upward transport of auxin. They did not interpret these curvatures as due to upward transport, either because they were not statistically significant or else because they were ascribed to diffusion in films of water

on the surface or in the intercellular spaces (Van der Weij). But it remains possible that they were genuinely due to a slight upward transport through the cells. Again it is possible that upward transport of auxin in coleoptiles takes place by some mechanism different from that of downward transport, and that this mechanism scarcely works in the isolated pieces of tissue. In any case the interest of the experiments with isolated pieces is not in any way diminished by the present results, since transport is certainly much more effective downwards,¹ and also since the mechanism may be different.

That the polarity of transport of auxin in stems is a very general rule follows from many observations on the downward spread of cambial growth. For it is well known that cambial growth as a rule (though there are exceptions) spreads only downwards from growing buds or leaves, and this spread of cambial growth is due to transport of auxin (Snow, 1935). But observations on cambial growth are necessarily made in the fully elongated parts, and in these the polarity may be more rigidly fixed than in the young elongating parts. It may be noted incidentally that in poplar roots Brown (1936) has been able experimentally to reverse the normal downward spread of cambial growth, but in similar experiments on the stems he could not do so.

SHORT AND LONG-TIME UPWARD EFFECTS IN PEA SEEDLINGS

It now became an urgent question how these results were to be reconciled with Miss Le Fanu's finding that in pea seedlings hetero-auxin paste *retards* the elongation of the parts above it. In the hope of obtaining a clue, some preliminary experiments were performed on pea seedlings grown in the shade. It was soon found that by placing a blob of hetero-auxin paste (1 in 1000) on one side of the stem just below a young developing internode, a strong negative curvature could be caused in the young internode above, indicating that the paste was *accelerating* growth in the parts above it, just as in the oats and sunflowers. But it was also noticed that after one day (or perhaps less) these curvatures did not increase any more, but diminished, even when the counteracting effect of geotropism was eliminated by bending the seedlings near the base so as to keep their upper parts vertical.

This observation suggested that the accelerating effect on the parts above the paste may come on first and be followed later by the

¹ Naturally this is not so when the auxin (or other growth substance) is introduced directly into the vessels, as in various experiments of Hitchcock & Zimmerman (1935).

retarding effect: for in Miss Le Fanu's experiments the first measurement was made after 24 hours, and it was not until the second day that the retardation became well marked. It was therefore decided to test the upward effect of hetero-auxin paste on pea seedlings by measurements of growth made after shorter intervals.

For this purpose the pea seedlings were grown in a greenhouse under overhead light only, being screened from the sides and from the sun. It was found best to sow the seeds unsoaked in soil. The ends of the zones to be measured were marked with tiny spots of Brunswick black, and they were measured to the nearest 0.25 mm., with occasional estimates to 0.1 mm. The leaves and internodes were numbered from below upwards, the first internode coming above the first leaf.

In a first experiment, performed in May, with ten seedlings matched in pairs for size, the young third internodes were marked for measurement when between 4.5 and 8 mm. in length, the total height of the seedlings being about 40 mm. Also the uppermost part of the internode below (the second internode) was marked, a zone ranging from 4 to 6 mm. in length. Immediately below this lower zone a ring of hetero-auxin paste (1 in 1000) was placed round the stem of one plant of each pair, the other plant of the pair serving as control. The leaves were removed from below upwards up to the young sixth leaf inclusive (which was about 1.5 mm. long), since the young leaves by secreting auxin diminish the effects of applied auxin (Thimann & Skoog, 1934; Le Fanu, 1936). Table I shows the mean growth of the two zones in experiments and controls in various successive periods.

TABLE I. Mean growth in millimetres of measured zones in successive periods. (The extremes are placed in brackets after the means)

	First 6½ hours	Next 16 hours	Next 24 hours	Next 48 hours
3rd internode:				
Plants with auxin paste below	1.4 (0.75, 2.5)	0.4 (0, 0.75)	1.0 (0.5, 1.5)	2.35 (1.25, 3.5)
Controls	0.5 (0.25, 1.0)	1.6 (0.75, 2.5)	3.65 (2.75, 4.75)	13.45 (11.0, 15.25)
Next lower zone:				
Plants with auxin paste below	0.95 (0.75, 1.0)	1.6 (0.75, 2.5)	0.35 (0, 0.75)	0.2 (0, 0.5)
Controls	0.15 (0, 0.25)	0.4 (0, 1.0)	1.15 (0.75, 1.75)	2.35 (1.0, 4.0)

From the table it can be seen that the hetero-auxin paste at first accelerated the elongation of the parts above it, but afterwards (by the second day or sooner) retarded it increasingly. It can also be seen

that the change over from acceleration to retardation took place sooner in the higher and more distant zone than in the lower zone—after 6½ hours in the former and after 22½ hours in the latter. It may be added that the growth of the young leaves of the terminal buds was also greatly retarded by the hetero-auxin paste below.

In another experiment with four pairs of plants, the third internodes were longer at the start—from 12 to 19 mm.—and the paste was placed in a narrow ring covering 2 or 3 mm. of the extreme base of this internode. The remainder of the internode, above the paste, was divided by a small mark into two nearly equal zones, and both were measured. Table II shows their growth in successive periods. The variability was less than in the previous table.

TABLE II. *Mean growth of measured zones in millimetres*

	First 9 hours	Next 15½ hours	Next 48 hours
Upper half of 3rd internode:			
Plants with auxin paste below	0·75	0·31	0·88
Controls	0·62	1·56	5·31
Lower half of 3rd internode:			
Plants with auxin paste below	1·05	0·62	0·44
Controls	0·38	1·5	4·01

It can be seen that the result was similar to that of the first experiment, except that in the upper zone the preliminary acceleration was not well marked, and in the lower zone the change over to retardation came sooner.

A third experiment, with three pairs of plants, was arranged similarly to the first experiment, except that the plants were kept in a very damp atmosphere under a bell-jar. The result was very closely similar to that of the first experiment, but it will not be concluded with certainty that the transpiration stream was not concerned in either of the upward effects, since probably transpiration was not completely stopped.

Acceleration followed by retardation was obtained by Zimmerman & Wilcoxon also (1935, pp. 217 and 220) in artichoke and tobacco plants, after applying various growth substances, including hetero-auxin, in lanoline to the stems "from the tip back to the base". The rate of increase in height was much accelerated on the first day, and afterwards retarded. But it seems to the writer that by covering the entire length of the stem with paste, they made the conditions more complicated and consequently the interpretation more difficult.

With regard to the preliminary acceleration of growth, it may

reasonably be considered that this is due to upward movement of the hetero-auxin itself, just as in the experiments with oat coleoptiles and sunflower hypocotyls. But the later effect, the retardation, which is the effect observed by Miss Le Fanu, is considered by her to be probably indirect and due somehow to promotion of growth elsewhere. She also considers that the correlative inhibition of lateral buds and shoots is probably a similar indirect effect, thereby adopting provisionally a theory which was proposed by Loeb (1924, pp. 101 and 108) and again by Laibach (1933). The present writer is inclined to accept this theory, but since experiments bearing upon it are in progress, it will not be fully discussed at present.

THE EFFECTS OF AUXIN IN THE TRANSPERSION STREAM

Hitchcock & Zimmerman (1935, p. 459) found that the growth of plants of tobacco and marigold, grown in pots, was retarded by adding to the soil small quantities of hetero-auxin, or of other substances whose effects are rather similar. The retardation of growth was apparently observed after several days. This treatment also caused epinasty of leaves and stems, and it was established that the growth substances travelled up from the soil with the transpiration stream, for they were able to travel up through a dead zone of stem and cause epinasty of leaves above (p. 463).

Independently Miss Le Fanu (1936) found that the elongation of young internodes of pea shoots cut off near the base was retarded if the shoots were placed with their cut bases in weak solutions of hetero-auxin, instead of in water, so that they drew up the hetero-auxin with the transpiration stream. She proposes the general rule that the auxins retard the growth of a young zone of stem if applied to a part morphologically below that zone, but accelerate its growth if applied to a part morphologically above it; and partly from this rule she infers that the retarding effect is probably indirect, the auxin itself travelling mainly downwards in the living tissues.

But the experiments with cut pea shoots do not fit in very well with this rule. For though it is literally true that in these experiments the hetero-auxin solution was applied near the base of the shoot, yet, since it was rapidly drawn up with the transpiration stream, the arrangement was physiologically equivalent to applying the hetero-auxin directly to every part of the shoot which the transpiration stream reaches, as Miss Le Fanu herself points out (1936, p. 209). It therefore occurred to the writer to wonder whether the retardation observed was really brought about in the same way as the

retardation by hetero-auxin applied externally in lanoline: and accordingly the following experiments with cut shoots standing in hetero-auxin solutions were performed to test whether the effects were entirely similar.

The concentration of the hetero-auxin solution was 1 in 2×10^4 , which was the highest concentration used by Miss Le Fanu. In one experiment eight pea seedlings, matched in pairs, were cut off near the base when their fourth internodes were only from 3.75 to 5.1 mm. long. They were deprived of their youngest leaves by being decapitated close behind the apex, but the young leaf at the top of the fourth internode was left to draw up the transpiration stream through it. Also two fully expanded leaves were left below to photosynthesize. The cuttings were kept in diffuse light, in a greenhouse in May, one member of each pair standing with its base in hetero-auxin solution, and the other in water. Table III shows the mean growth in successive periods.

TABLE III. *Mean growth of fourth internodes in successive periods, in millimetres. (The extremes are given in brackets when the means are close)*

	First 9 hours	Next 16 hours	Next 28 hours	Next 48 hours	Next 48 hours
Shoots with bases in auxin solution	0.09 (0, 0.2)	0.14 (0, 0.25)	0.48	1.75	0.37
Controls in water	0.14 (0, 0.25)	0.25 (0, 0.5)	1.62	4.37	4.62

It can be seen that the young internodes of the cuttings standing in the solutions were very strongly retarded, and further that they were not first accelerated during the first 9 or 24 hours, as were the internodes which had hetero-auxin paste applied below them.

In another experiment, the treatment was the same, but the young fourth internodes were longer, those of the cuttings in the solutions (three in number) ranging from 8.25 to 9.25 mm., and those of the controls (four in number) from 6.75 to 10.25 mm. Table IV shows the result arranged as before.

TABLE IV. *Mean growth of fourth internodes in successive periods, in millimetres. (The figures given in brackets are the extremes)*

	First 8 hours	Next $22\frac{1}{2}$ hours	Next 30 hours	Next 48 hours
Shoots with bases in auxin solution	2.58 (1.5, 3.25)	4.25 (2.25, 5.5)	4.58	7.25
Controls in water	1.19 (1, 1.5)	1.31 (1.25, 1.5)	3.75	8.56

It can be seen from the table that, when the measured internodes were so long as 8.25 or 9.25 mm. at the start, they were not retarded at all by the solution, not even after 4 or 5 days. Indeed during the first 30 hours they were quite strongly accelerated. This is a second striking difference between the effects of hetero-auxin when drawn up with the transpiration stream and when applied below externally in a paste. For in the experiments with the paste already reported internodes much longer than these were strongly retarded, after the preliminary acceleration. The results therefore strongly suggest that retardation by growth substances in the transpiration stream is a quite different phenomenon, to which no attempt need be made to apply the "indirect" theory of retardation by auxin.

The hetero-auxin had much more violent effects when drawn up with the transpiration stream than when applied in lanoline, for it caused the leaves to curve in various ways and the internodes which were fairly long but still growing to bend right over. It also made these internodes excessively pliable, so that it was exerting on them the typical effect which regularly accompanies acceleration of stem growth by auxin. After several days these internodes finally became rigid.

The stage of growth at which the young internodes change their reaction to hetero-auxin in the transpiration stream and become liable to be accelerated by it instead of being retarded, is the stage which normally comes just before they start their rapid elongation; and this fact makes the change in reaction seem less surprising. On the other hand, when the auxins are applied externally to young zones of stem or coleoptiles, they tend to accelerate the growth of the parts below (so far as is at present known) however young they may be.

Naturally the retardation by auxin in the transpiration stream might be studied much more thoroughly, but since it appears to be a different phenomenon from retardation by auxin paste, this would be outside the scope of the present paper.

SUMMARY

1. By applying a paste of hetero-auxin in lanoline near the bases of decapitated dark-grown oat coleoptiles, on one of the narrow sides, strong negative curvatures are caused in the parts above the paste in about 6 hours or less.

2. When the paste is applied to one of the broad sides near the base the curvatures above are smaller, though distinct, and develop more slowly.

3. It follows that, contrary to the general belief, hetero-auxin can be transported to some extent in the morphologically upward direction in coleoptiles, and that this upward transport takes place largely, but not entirely, in the conducting strands. In whichever direction the hetero-auxin is transported in coleoptiles, it accelerates their growth.

4. In decapitated dark-grown sunflower hypocotyls also the same paste applied to one side near the base causes negative curvatures above within 24 hours, indicating acceleration of growth.

5. When pea seedlings have a ring of the same paste put round the stem close below one of the growing internodes, the elongation of this internode is at first accelerated (for less than 24 hours) and then strongly and increasingly retarded. The preliminary acceleration is presumably due to upward-moving hetero-auxin, and the subsequent retardation, which is the effect found by Miss Le Fanu (1936), may be indirect. The young leaves of the terminal bud are also retarded.

6. When pea shoots, cut off at the base, are placed with their bases in a hetero-auxin solution ($1 \text{ in } 2 \times 10^4$) instead of in water, the young internodes above are very strongly retarded, as was found by Miss Le Fanu (1936), but only if they are not more than about 5 mm. long at the start (when grown in the same conditions as the writer's plants). They do not show any preliminary acceleration. If, however, they are about 8 mm. long, or more, at the start, they are never retarded at all, and for the first day or two they are even considerably accelerated. The longer growing internodes become very pliable.

7. From these differences it is concluded that the retardation of very young internodes by hetero-auxin drawn up with the transpiration stream is probably brought about in a different way from retardation of internodes by hetero-auxin paste applied externally below them.

8. The results are discussed in the text and compared with those of other workers.

The writer is much indebted to Dr R. Weissberger for kindly synthesizing the hetero-auxin used in these experiments.

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DEVELOPMENTAL STUDIES OF THE PINE-
APPLE *ANANAS COMOSUS* (L) MERR.

I. ORIGIN AND GROWTH OF LEAVES AND
INFLORESCENCE¹

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(With Plate IV and 6 figures in the text)

THE origin and early growth of seedling pineapple plants have been discussed by Miles Thomas & Holmes (1930). As is the rule for monocotyledons a single leaf is first formed followed in succession by the origin of younger leaves higher up on the axis of growth.

In the present report we are concerned with the origin of new leaves in vegetative reproduction and the phenomena involved at the apex of the plant during the transition from leaf production to the formation of an inflorescence. In a mature plant the axis or main stem of the plant is terminated by the inflorescence which develops into the pineapple fruit.

With the cessation of vegetative growth at the apex due to the formation of the inflorescence, axillary vegetative growth takes place giving rise to a number of lateral shoots which are the principal means of propagation of the pineapple.

The observations reported here were made on paraffin and free-hand sections from the apical meristem of plants of the Cayenne variety.

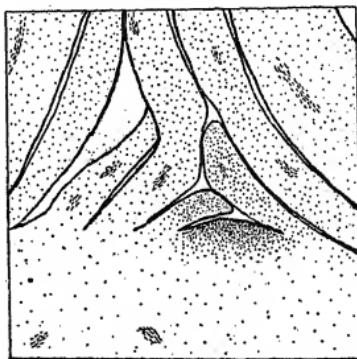
LEAF DEVELOPMENT

The apical meristem of a plant producing only leaf growth is characteristically small in comparison with the cross-sectional area of the main stem at the apex of which it is centrally located. In shape, the meristem area is circular and slightly convex.

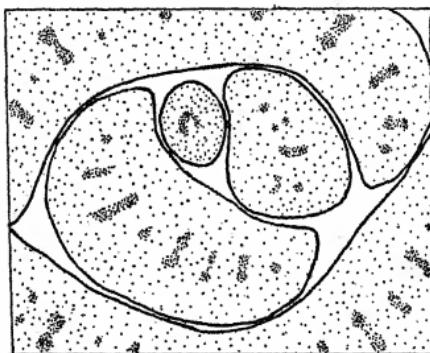
The first visible evidence on this apex of the beginning of a new leaf is a small bulge or ridge composed of a number of cells thrown up

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at one edge of the circular meristem area. This protuberance of the meristem cells increases in size and elongates into an embryonic leaf (Text-fig. 1). After this primordial leaf has advanced in growth for a



Text-fig. 1. Longitudinal median section through the growing point of a plant producing only leaves. The meristem area is shown. $\times 45$.

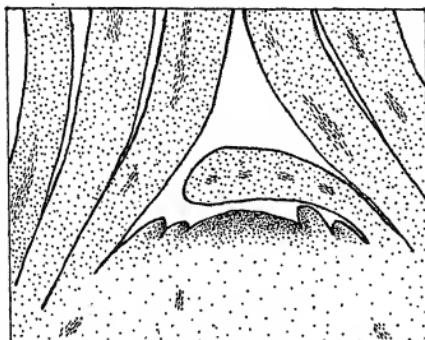


Text-fig. 2. Cross-section just above the meristem area and showing the relative size and position of the three youngest leaves. $\times 90$.

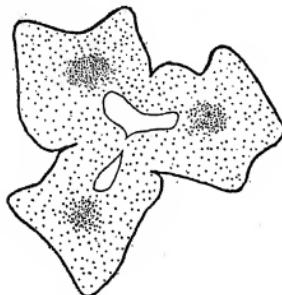
short time the next younger leaf primordium becomes evident some distance away around the circumference of the circular growing point.

During the normal growth of the plant it appears that the time interval between the origin of successive leaves is relatively uniform,

as is also the angular distance between successive leaf primordia. The constancy of the relative sizes of the three young leaves is an evidence of this fact. The several youngest leaves at the growing point appear to have a constant relative cross-sectional area such that each is



Text-fig. 3. Section similar to Text-fig. 1 but from a plant beginning to form an inflorescence. The larger meristem area is clearly shown. $\times 45$.



Text-fig. 4. Cross-section of a style showing the three-lobed shape and the stylar canal. $\times 72$.

about one-third the size of the next older leaf (Text-fig. 2). It is this time and space relationship which determines the type of phyllotaxy of stem and fruit. This subject of the phyllotaxy will be discussed in a later section of this paper. Growth takes place at the base of the leaf at its point of attachment to the main stem, the leaf tip, therefore, being the oldest part of the leaf.

THE ORIGIN AND DEVELOPMENT OF THE INFLORESCENCE

During a part of the growth season of 1934 and 1935, twenty-five plants were removed each week from a normally growing field. The growing point of each plant was sectioned both freehand and by use of the microtome and examined under a microscope in order to study the morphological changes taking place when the apical meristem ceased to produce foliage leaves and began to form an inflorescence. The numerical results of these observations are recorded in Table I A.

The first evidence of this change in type of growth was seen in the marked increase in the diameter of the meristem area. This is shown clearly by comparing Text-figs. 1 and 3. This change takes place rapidly and uniformly in plants of comparable age and health. In the series of twenty-five plants removed 17 December 1934, all had only the typical leaf producing meristem, but in Series 3, removed 1 week later, twenty-three of the twenty-five plants possessed the wide meristem area indicative of the beginning of the inflorescence.

The growing point meristem reaches its greatest diameter when the first rows of flowers or "eyes" are formed. Measurements of the width of the apical meristematic area (α of Pl. IVB) of each of the twenty-five plants removed weekly in 1936 showed a marked inverse correlation between the rows of eyes formed and the average width of the apical meristem. This relation apparently limits the number of florets (eyes) which can be formed on a single fruit, for when the reduction in meristem diameter reaches certain limits, leaf primordia replace flower primordia.

Following the enlargement of the meristem area, flower-bud primordia are produced as bulges at the margin of the meristem similar to the origin of leaf primordia. The first organ to appear on the margin of the meristem with increased diameter is the bract which subtends the pineapple flower. This is followed in rapid sequence by primordial ridges of the sepals, petals, stamens and pistil.

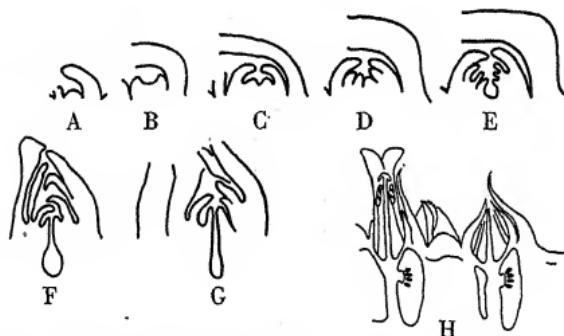
In Text-fig. 5, successive stages in the development of a single flower are shown in semi-diagrammatic drawings. In this set of drawings, A shows the primordia of the bract which will later subtend a single flower. B shows two of the three sepal primordia which appear adjacent to and above the bract. In C two of the three petal primordia are shown starting adjacent to the sepals, and in D stamen primordia make their appearance. In E we see the beginning of the carpel structure, further developments of which are shown in F, G and H.

TABLE I A
Number of plants showing different stages of flower development

Plants started October 1933	Series	Foliage leaves only	Wide growing point	Number of rows of florets beginning to develop	Stage of development										Degree of redness	Crown open (in.)																
					1	2	3	4	5	6	7	8	9	10	11	A*	B	C	D	E	F	G	H	A	B	0	1	2	3	4		
10. xii. 34	1	25
17. xii. 34	2	25
24. xii. 34	3	2	23
31. xii. 34	4	.	2	12	8	3
7.i. 35	5	.	.	.	2	7	11	1	2	3	1	
17.i. 35	6	2	4	3	5	6	4	.	1	.	3	9	9	3	2		
21.i. 35	7	1	2	3	4	9	4	.	.	3	7	9	5		
28.i. 35	8	2	.	8	4	20	14	11	
4. ii. 35	9	1	.	2	.	1	.	.	.	5	11	8	18	2	4	11	5	5	.	9		
11. ii. 35	10	10	13	.	.	1	2	13	10	23	
18. ii. 35	11	1	.	.	.	1	.	18	19	12	4	3		
5. iii. 35	12	19	19+	4	5	7	

TABLE I.B. Correlation between rows of eyes formed, peduncle height and width of the growing point meristem

Peduncle height mm.	Rows of eyes originated	Average width of growing point mm.	Average stage of oldest eye
None	None	0.361	No eyes
None	Wide growing point	0.549	
3.6	1 row	0.656	Basal eye tissue
7.1	2 rows	0.643	Forming sepals
8.6	3 "	0.649	" "
12.2	4 "	0.596	petals
16.1	5 "	0.556	" "
20.8	6 "	0.515	carpels
23.6	7 "	0.509	" "
28.0	8 "	0.442	" "
31.6	9 "	0.402	" "
43.2	10 "	0.328	ovules
47.1	II " and all	0.227	" "
56.0	All	0.234	" "



Text-fig. 5. Diagrams showing the development of the pineapple flower and fruitlet. A, the floral bract primordia; B, sepal primordia; C, petal primordia; D, stamen primordia; E, carpel primordia; F, enlargement and closing up of the carpel; and H, mature flower, and a non-median section through another.

The period of time from the beginnings of the first flower until the complete formation of the last flower of the inflorescence is approximately 3 weeks. The number of flowers formed varies with the size and vigour of the plant. Healthy and normal-sized plants develop about 150 flowers on the first inflorescence produced by a single plant.

The flower parts originate in sets of three: three sepals, three petals, six stamens (two groups of three) and three carpels. The members of each set grow simultaneously and the different sets appear in succession. During growth, the sepals and petals extend upwards

at an acute angle with the receptacle and thus produce, in form, a double tent enclosing the essential flower organs. All the flower parts appear to originate at almost the same level and grow away from this point. The central portion of the growing point around which these flower parts are grouped does not appear to share in this growth but remains at the original level. The growth of the surrounding parts thus forms a cup or cavity. The outer flower parts which are older appear to grow at a faster rate than the inner parts, thus causing the walls of the cavity to converge at the top. The growth and development of the carpels then fill this cavity, becoming folded in a characteristic fashion.

The apex of each carpel grows upwards between the stamens and there fuses into a single unit which becomes the style of the pistil having the three stigma lobes as its apex. Extending through the centre of the style is a three-lobed canal down which the pollen tubes grow to effect fertilization. Text-fig. 4, a cross-section of the style, shows this stylar canal.

During growth the edges of the three carpel primordial leaves are folded towards each other and come to occupy the cup cavity at the centre of the point of origin of the flower parts as mentioned above.

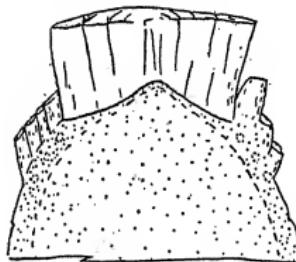
The edges of these carpel primordia not only come together during growth but are forced to curl back upon themselves, causing the edges to be turned in opposite directions. When formation of the carpels is completed, this twin nature of the placental area is not so evident. In Pl. IV A, which is a tangential section of a young fruit, we can see the indistinct line where the walls of the carpels have come together. The position of the ovules is evidence that the edge of the carpel leaf had turned in towards the centre of the leaf during development of the carpels.

During carpel development the sides where two carpels meet sometimes fail to close up completely and leave a small, narrow, elliptical opening extending downwards between the carpels, from the bottom of the blossom cavity of the cup, permitting air to enter these spaces. As the fruit develops to maturity the cells of the carpel walls thus exposed to air become dark coloured and hardened. Black spots of this origin are illustrated in Pl. IV A. In this figure it is possible to see the point where black spots originate in the failure of the carpel leaves to form a perfect contact. This blemish is much more prevalent in fruits of the Queen and Ruby varieties than it is in Cayenne.

Since the transition from a leaf producing meristem to a flower-bud producing meristem is abrupt, and the appearance of flower-part

primordia follows immediately after the increase in diameter of the growing point, it becomes obvious that the leaves, fifteen to twenty-five in number, which are later found on the peduncle, were all initiated before the beginning of the inflorescence although there was no peduncle evident at that time.

Within about 5-6 days after this change at the growing point, evidence can be seen that the peduncle elongation has started by a pronounced elevation of the central portion or apex of the stem of the plant (Text-fig. 6). As the development of the inflorescence proceeds, the elongation of the peduncle also proceeds and has apparently reached its full length by the time flowers commence to open.



Text-fig. 6. Median longitudinal section of the growing point showing the beginning of the peduncle growth by the elevation of the central area. $\times \frac{4}{3}$.

Whether the altered appearance of peduncle leaves and the short leaves at the base of the fruit (involucral leaves) is due to arrested development of foliage leaves or to a change in mode of development is a question to which no answer is apparent.

The last peduncle leaves to be produced before flower-bud formation starts develop a characteristic back fold or kink at the tip which is apparent as late as flowering. This kink is clearly shown in Pl. IVB.

When the inflorescence is completed the meristem area at the growing point returns to a leaf-producing condition but less abruptly than the opposite change. Following the return to a small leaf type of growing point the group of short leaves known as the crown or top is produced, which terminates the axis of that particular plant.

Coincident with completion of the flower formation the involucral and peduncle leaves assume a pink and later a reddish colour. When this pink coloration can be found in these leaves it can be taken as the indication that flower formation has been completed, and that crown development is taking place.

The nature of the stimulus which causes the change in meristem type is unknown. It has been shown, however, that the plant or young shoot can be treated at any time during its growth with certain unsaturated hydrocarbons, and that these cause the foliar type of meristem to change abruptly to the flower-bud producing type; the plant then proceeds to form a fruit which in size is largely proportional to the size and age of the plant when treated.

From the study of fruit development it has been possible to determine the approximate length of time required for several different stages of fruit development to be completed. The periods of time given in Table II below are the averages from a number of plants observed during two successive years.

TABLE II. *The time required for completion of different stages of the development of a pineapple inflorescence and fruit*

Periods of fruit development					Average time duration days
(1) From planting to beginning of inflorescence	427
(2) From beginning to end of inflorescence formation	37
(3) From end of inflorescence formation to first open flower	43
(4) Period of flowering	26
(5) From last open flower to ripe fruit	109
(6) Total period of fruit development	215
(7) From planting to mature fruit	642

THE ORIGIN AND GROWTH OF SLIPS

Slips are branches of the main stem which develop in the axils of the peduncle leaves. They usually have a small, vestigial, fruit-like structure at their base through which the slips are attached to the peduncle.

At a very early stage of leaf development it is possible to see an island of meristem cells from which slip buds on the peduncle and shoot buds on the main stem are developed. Examination of the peduncle at the stage when the flower-bud formation is just completed has shown slip buds to be present on the lower or older portion of the peduncle. The slip buds then originate some time previous to the appearance of the red coloration of the leaves in the centre of the plant.

PHYLLOTAXIS OF THE PINEAPPLE

The pineapple exhibits the spiral type of phyllotaxy in regard to both the leaves and flowers.

Miles Thomas & Holmes (1930) from a study of angular distance between leaf primordia of seedling pineapple plants stated that the

phyllotaxy was 11/18. In this system of expressing the phyllotaxy of a plant the numerator of the fraction indicates the number of spiral turns about the axis and the denominator the number of leaves in this spiral until one is vertically above another and older leaf in its attachment to the stem.

We, however, have not been able to confirm these authors' findings in the phyllotaxy of the more nearly mature plant.

In the pineapple the central point of each leaf on the main axis is marked by the presence of a small dormant axillary bud. After leaves have been removed from a plant these small buds remain on the stem and serve as markers for the midpoint of each leaf.

The leaves were removed from a number of normal plants and a vertical line inscribed on the axis beginning at one of these dormant buds near the butt end of the main stem. It was noticed that this line with great regularity bisected the 14th and 27th buds. This indicated an interval of thirteen buds before another appeared vertically above bud number one. Tracing the buds around from Nos. 1 to 14 there were five complete turns about the axis. These findings therefore establish the leaf phyllotaxy as 5/13.

TABLE III. *Data from which the leaf phyllotaxy of the pineapple plant was determined*

	Position of the 14th bud (leaf) with respect to the 1st		
	Vertically above	Slightly to the right in right spiral plants	Slightly to the left in left spiral plant
No. of plants	10	1	1
Position of 27th bud	27th above 14th	27th to the right	27th to the left
No. of plants	2	4	1

The deviation to the right or left in right and left spiral plants of the 14th and 27th buds from the vertical line is possibly due to a slight torsion of the axis during growth. This explanation for these deviations appears more plausible from the fact that in each case the deviation was in the same direction as the leaf spiral.

Preliminary observations of the leaf arrangement on the pineapple plant stem were made by Sideris (1926) in the course of studies of the fibrovascular system of the plant. While he did not state what type of phyllotaxy was present it was clear from his analysis of the fibrovascular system of the stem and successive leaves that the 5/13 type was present.

Priestley *et al.* (1935) have studied in detail the phyllotaxy of a monocotyledon in relation to the internal arrangement of the fibro-

vascular system and the origin of new leaves at the meristem area. They discuss the possibility of a transition from a simple type of phyllotaxy to a more complex one, as being the logical result of an increase in the size of the meristem area. We have shown in the pineapple that such a change is the first visible evidence of the formation of the inflorescence, which we show has a phyllotaxy one degree more complex than the phyllotaxy of the stem bearing only leaves.

The phyllotaxy of the inflorescence has been found by Dr M. B. Linford of this Station (unpublished data) to be of the $8/21$ type and is thus different from that of the leaves on the same plant.

Plants of the Cayenne variety were found to have either right- or left-hand spirals and the fruits were of the same type as the main stem of the plant. Whether the shoots produced by a plant have right- or left-hand spirals appears to be a matter of chance, depending on whether the second leaf primordium develops on the right or the left side of the initial primordium.

The investigation concerning phyllotaxy has been carried on using the Cayenne variety.

An examination of pineapples growing in the variety garden has shown that the large fruited varieties, Queen, Taboga, Pernambuco and Wild Kailua, have the same phyllotaxy as Cayenne. The smaller fruited pineapples, *Ananas microstachys* Lindm. and *A. microcephalus* (Bak.) Bertoni, have a fruit phyllotaxis of $5/13$ and a phyllotaxis of $3/8$ for the plant.

The F_1 hybrids produced by crossing *A. comosus* L. (Merr.) variety Cayenne with *A. microstachys* show a segregation of fruits, some being like Cayenne and some like *A. microstachys*.

The phyllotaxy of the pineapple is not, however, a fixed genetic character but appears to be dependent upon the size or area of the meristem at the growing point. We have already shown that the meristem area increases in size as the first visible steps in formation of the inflorescence.

Evidence supporting this conclusion is to be found in the fruits produced by plants forced to flower prematurely. It has been known for a long time that pineapples could be forced to flower by smudging with smoke. Rodriguez (1932) in searching for a constituent of smoke which was responsible for this result found that ethylene gas was effective. Later the senior author found that acetylene was equally efficient in forcing flowering. Using the latter gas, Cayenne plants of different ages have been forced to flower and form fruits. It was found that small plants up to 2 months of age when treated

produced fruits having the $5/13$ fruit phyllotaxy, but that plants treated when 4 months of age or older produced fruits having the normal $8/21$ phyllotaxy.

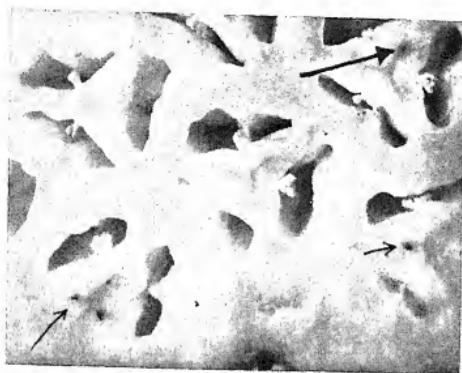
We have called attention to the increase in the size of the meristem area preceding the development of the inflorescence and a reduction at the completion of flower formation and beginning of the crown development. These two transitional zones are marked in the mature inflorescence and fruit by a series of small short leaves, the involucral bracts at the base of the fruit and sterile bracts at the top between the fruit and the crown. These leaves mark the transition from a $5/13$ phyllotaxy to the $8/21$ phyllotaxy of the fruit and back again to the $5/13$. The first change, we believe, is quite abrupt and rapid but the second one much less so.

These two periods may be looked upon as critical ones in the development of fruits. Abnormalities of fruit and crown development such as double monsters, fasciations and multiple crowns may be the results of interference with the regular transition from one to the other of these regular systems of phyllotaxy. Multiple crowns may be the result of the failure of the meristem area to return completely to the $5/13$ type following the $8/21$ type of the fruit.

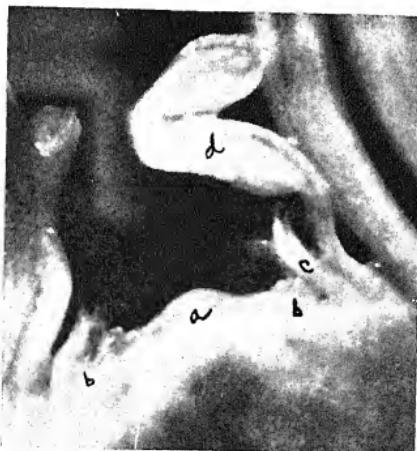
The normal phyllotaxy of the plant and fruit appears to rest upon a co-ordinated space relationship in the meristem area. It is quite probable that chemical and physiological factors play an important role in bringing about these size changes in the meristem, but we are not at this time attempting to visualize their place in these phenomena.

SUMMARY

1. The pineapple plant is a monocotyledon with a terminal inflorescence. The first visible evidence that an inflorescence is about to be formed is the increase in the size of the meristem area at the apex of the plant axis.
2. The average period of time for the plant to complete inflorescence formation and growth and the several divisions of these processes have been determined.
3. The inflorescence begins development before the peduncle starts elongation.
4. The phyllotaxy of the large fruited varieties is $5/13$ for the plant, and of the fruit, $8/21$. Small fruited varieties have a $3/8$ plant phyllotaxis and $5/13$ for the fruit.
5. The time when the phyllotaxy changes from that of the leaf-

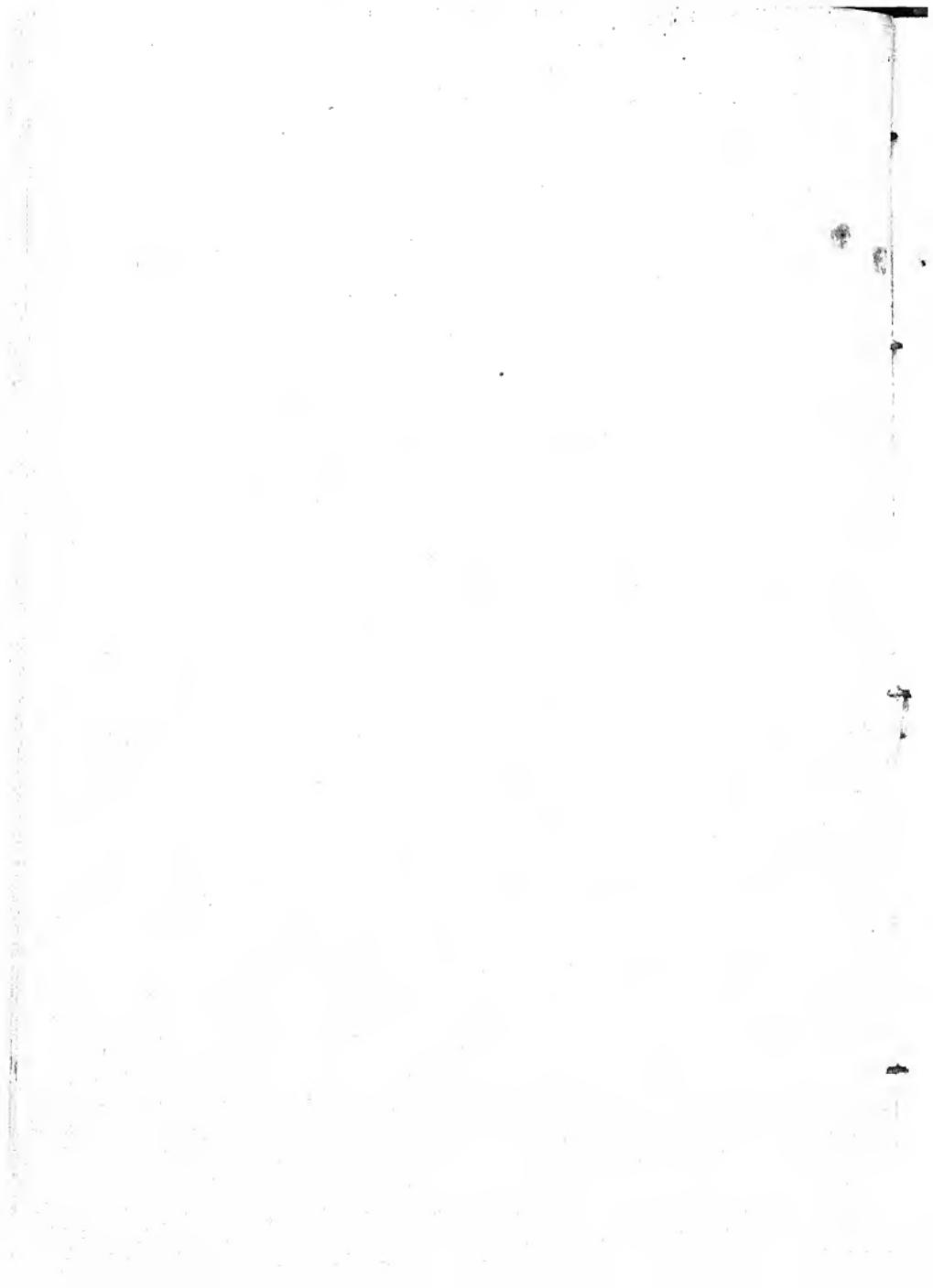


A



B

KERNS, COLLINS & KIM—DEVELOPMENT OF PINEAPPLE



producing growing point to the inflorescence growing point is considered a critical one for the production of fruit and crown abnormalities.

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EXPLANATION OF PLATE IV

A. Tangential section of a young pineapple fruit showing the carpels, ovules and intercarpellary fissure, the latter indicated by arrows. $\times 1$.

B. The stem growing point at the beginning of inflorescence development showing: *a*, the meristem area; *b*, the first flower bud; *c*, the floral bract subtending the first flower; and *d*, the last involucral leaf showing the characteristic fold at its tip. $\times 45$.

FLORAL ANATOMY AND PHYLOGENY IN THE RUTACEAE

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(With 1 figure in the text)

IN my studies on the floral anatomy of the Leguminosae I have found that the larger groups within the families—the tribes—each possesses a very uniform floral anatomy which may be correlated with characters of external morphology (Moore, 1936). The Papilionaceae have yielded only three basic patterns of floral anatomy, while the Caesalpiniaceae are characterized by two distinct types, one of which is not found amongst the Papilionaceae.

When Miss Saunders's account of the Rutaceae appeared (1934), I was quite eager to examine it in the light of the situation in the Leguminosae. Did the Rutaceae show a uniformity of floral anatomy that could be linked with the basic divisions used in current taxonomic treatment of the family? If there were discontinuities in floral anatomical types, could these breaks be associated with intrafamilial phylogeny? Could floral anatomy give a clearer picture of the evolutionary progress and processes within the Rutaceae?

The careful work by Miss Saunders (1934) on the floral anatomy of the Rutaceae is the entire basis for the following discussion. It provides an excellent summary of the general facts of floral organization in eighteen representative genera, presenting sufficient detail to enable one to reconstruct the general vascular anatomy of the flower. Miss Saunders has emphasized the polymorphic interpretation of the gynoecium. I disagree heartily with the polymorphic interpretation as it has been applied in the Rutaceae. The "basic principles" on which this interpretation rests have been ably refuted by Eames (1931).

I do wish to emphasize the phylogenetic significance of the facts of floral anatomy which Miss Saunders presents for the Rutaceae. These facts are overshadowed by the extended effort to explain the gynoecium in terms of carpel polymorphism. The significant points in the floral anatomy of the Rutaceae are found in the arrangement

of the traces to the other floral parts. In my diagrams, which are constructed after Miss Saunders's drawings of transverse sections and textual description, I have omitted the carpel traces. Carpel number varies within the family with five as a common number; four, three and two carpels are found in some genera; while a few genera have ten or more carpels.

The present systematic arrangement of the Rutaceae (Engler, 1931) is based primarily on carpel number, fruit structure, glandular histology, and habit. We shall first examine the floral anatomy of the family, and then apply the results of the study to taxonomy and current phylogenetic ideas concerning the group.

Among the eighteen genera which Miss Saunders has examined in detail, there occur eight different sorts of anatomical conditions. These are illustrated in Fig. 1. They differ in adnation of traces, cohesion of traces, insertion of stamen traces, and in insertion of disc traces. These eight types are identical in the fact that the calyx trace and the trace of the opposed stamen are never adnate. The antesepalous stamen trace is usually alone, or it may be fused with one of the disc strands. The calyx laterals may be absent, they may depart from the calyx dorsal trace, or they may be fused with the corolla trace. The corolla trace may be free from all the others, it may be fused with the calyx laterals, it may be adnate to the stamen trace or to the stamen-disc trace. The antepetalous stamen trace may be free, it may be fused to the disc trace, it may be fused with the corolla trace, or it may be adnate to both corolla and disc traces. When numerous, the disc traces leave the stele independently; when the same number as the stamens, they are fused with the stamen bundles.

The simplest condition among the Rutaceae without a vascularized disc, is illustrated in Fig. 1A. Traces to calyx, corolla, and stamens are independent at all times. This is the case in *Diosma succulenta* Berg., *Xanthoxylum fraxineum* Willd., *Toddalia aculeata* Pers., *Feronia elephantum* Correa, *Aegle sepiaria* DC. The first two species are members of subfamily Rutoideae; *Toddalia* is in subfamily Toddalioideae; and *Feronia* and *Aegle* belong to the Aurantioideae.

In another member of the Rutoideae, *Cneoridium dumosum* Hook., B is seen, a more complicated condition in which the calyx laterals are fused to the corolla trace and the corolla trace to the opposed stamen trace.

In the disciferous Rutaceae, the simplest condition is found in *Ruta* (Rutoideae) and *Triphasia* (Aurantioideae). There is no adnation between the traces of the floral cycles (C). The calyx trace gives

rise to its laterals; the corolla, stamen, and the numerous disc traces arise independently.

The situation illustrated in D is found in *Ptelea* (Toddalioideae) and *Citrus* (Aurantioideae). Here, calyx and corolla traces are free

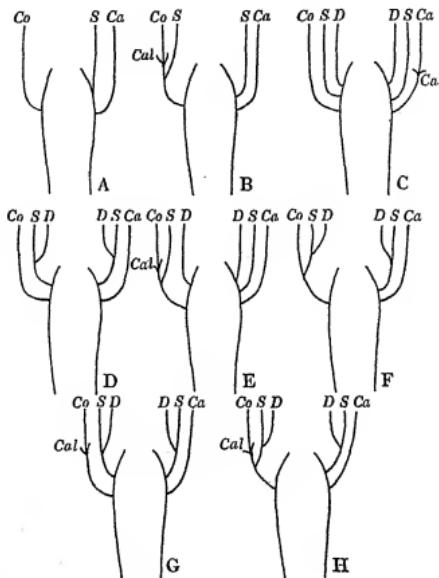


Fig. 1. Diagrams showing the vertical relationships of the vascular traces in the flowers of various Rutaceae. Reconstructions made from the data of Miss Saunders (1934). List of abbreviations used: *Ca*, calyx trace; *Cal*, calyx lateral trace; *Co*, petal trace; *D*, disc trace; *S*, stamen trace. A. In *Diosma succulenta* Berg., *Xanthoxylum fraxineum* Willd., *Toddalia aculeata* Pers., *Feronia elephantum* Correa, *Aegle sepiaria* DC. B. In *Cneoridium dumosum* Hook. C. In *Ruta bracteosa* DC., *Ruta graveolens* L., *Triphasia trifoliata* DC. D. In *Ptelea trifoliata* L., *Citrus Aurantium* L. E. In *Adenandra uniflora* Willd., *Barosma crenulata* Hook., *Calodendrum capense* Thunb. F. In *Agathosma imbricata* Willd. G. In *Boronia fastigiata* Bartl., *Boronia heterophylla* F. Muell., *Boronia megastigma* Nees. H. In *Dictamnus Fraxinella* Pers.

from the stamen traces, while the disc traces branch from the stamen traces.

In *Adenandra*, *Barosma* and *Calodendrum*, all Rutoideae, the disc traces are free from the stamen traces, but the antesepalous stamen, corolla and calyx lateral strands are fused (E).

There are no calyx laterals in *Agathosma* (Rutoideae). The disc

traces are fused to the stamen traces, and the antepetalous stamen trace to the petal strand (F).

The condition observed in *Boronia* (Ruteae) differs from *Agathosma* in that there is no fusion of antepetalous stamen trace, and in that the corolla trace and the calyx laterals are fused (G).

In *Dictamnus*, calyx laterals, corolla, stamen and disc traces on the same radius are adnate, while the antesepalous stamen trace is adnate to that of the disc (H).

The distribution of floral anatomy in systematic position is summarized in the classification given below. The same lettering is used as in the diagrams:

Subfamily Rutoideae: AA, B, C, EEE, F, G, H.

Tribe Xanthoxyleae: A.

Ruteae: B, C, H.

Borneoiae: G.

Diosmeae: A, EEE, F.

Cusparieae.

Subfamily Dictyolomatoideae.

Flindersioideae.

Spathelioideae.

Toddalioideae: A, D.

Aurantioideae: AA, C, D.

Rhabdodendrioideae.

The above table demonstrates the heterogeneity of floral anatomy in the subfamilies and tribes of the Rutaceae. If we take floral anatomy as a criterion of relationship, it is apparent that we must assume that the Rutoideae, at least, are highly complex phylogenetically. Seven of the eight types, ranging from the simplest to the most specialized, have been observed in this subfamily alone. In *Dictamnus* (H) and *Ruta* (C), two otherwise closely allied genera, there is greater divergence of floral anatomy than has yet been found in the other tribes.

The Aurantioideae present the least complex situation, while the other groups as far as known present more complex conditions. The Aurantioideae seem closer to the Toddalioideae as they both have the D type of anatomy in the flower, a condition not found in the Rutoideae.

Type C probably represents the primitive condition; type H, the most specialized; while type A is more than likely the result of reduction.

Vascular anatomy raises the question of the interpretation of the disc in this family. It is common knowledge that the hackneyed term "disc" has a protean connotation. Does the fact that one group of genera exists in which numerous disc strands are independent of the other traces, indicate that the disc may be interpreted in terms of a series of sterile carpels? In the other series of genera where stamen and disc traces are fused is the interpretation of the disc to be staminal? This second type of disc might be interpreted by assuming that the androecium once consisted of branched fertile members, the inner branches of which became sterilized.

These observations show that the present classification of the Rutaceae is one which runs directly across the lines of specialization in floral anatomy. Too few chromosome counts have been reported from the family to make possible a cytological test of affinities within the group. Bärner's (1927) observations on serodiagnosis agree remarkably well with those of floral anatomy. He observed a strong positive reaction between *Ruta chalepensis* L. and *Citrus Aurantium* L., forms which have a rather similar floral anatomy (types C and D), and are generally placed rather far apart by the taxonomists' classification. Between *Ruta chalepensis* L. and *Dictamnus albus* L. the reaction was only slightly positive, an observation strictly in accord with floral anatomy, but disagreeing with the taxonomists' assignment of *Ruta* and *Dictamnus* near to one another.

Is the pure taxonomist right in selecting fruit structure, carpel number, glandular histology, and habit as the criteria for delimiting the larger units in the Rutaceae? Are there other characters which would circumscribe the larger units of the family on the basis of possible relationships indicated by floral anatomy? Would an exhaustive study of all the characteristics of the family show up some which would correlate floral anatomy and other more subtle morphological characters the use of which might lead to a more natural arrangement of the assemblage? Would a critical study of floral anatomy in the Rutaceae give us a clearer, more accurate picture of the direction of phyletic change within the group?

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EXPERIMENTAL TAXONOMY

I. EXPERIMENTAL GARDEN TECHNIQUE IN RELATION TO THE RECOGNITION OF THE SMALL TAXONOMIC UNITS

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(With 3 figures in the text)

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I. THE PROBLEM OF EXPERIMENTAL TAXONOMY

EXPERIMENTAL studies of plants, both in the field and in the laboratory, have yielded information which appears to various investigators to bear directly on the nature and delimitation of taxonomic units. The classification of organisms on the basis of these experimental facts has been termed *Experimental Taxonomy*. Some doubt may exist as to whether the newer methods may supplant or enrich the existing system of taxonomy, but an examination of the scope of each plainly shows that they should be complementary and mutually helpful. Orthodox taxonomy is concerned with the convenient tabulation of morphological differences; as far as possible the arrangement is natural and it is the only method yet devised that can state to what part of the plant kingdom a dried specimen may belong. Experimental taxonomy fully appreciates the value of

morphological differences—in fact the cytologist has disclosed a fresh field for such investigation—but it also seeks to show the causes which underlie these differences, and to ascertain their physiological, ecological, or genetical nature. The species unit of orthodox taxonomy often includes minor units, which exhibit various degrees of morphological differentiation, regardless of whether such degrees have similar biological significance. Experimental taxonomy, on the other hand, transfers the emphasis from the species unit to the local race (see ecotype concept, Turesson, 1922): it is an attempt to classify evolutionary groups as they occur in Nature. On an extensive scale, as when the flora of a new region is being explored, the existing methods of taxonomy are undoubtedly those that would be employed. Experimental methods, however, would afford a means of probing more deeply into the nature of plant groups such as species of economic importance and others likely to yield valuable data relating to problems of evolution. A system of experimental taxonomy would make readily available this detailed information to botanists studying the phylogeny, distribution and ecology of plants.

Data relative to experimental taxonomy have been accumulating recently, and it has become necessary to consider how they should be documented so as to be available for reference. There are two alternatives: (1) to attempt to incorporate the findings of the experimental taxonomist, perforce relating mainly to the delimitation of the specific and subspecific units, within the framework of the existing system; and (2) to develop a separate but complementary system. Under present conditions the first alternative would add to the existing confusion, for the term species would have to cover not only the *linneon* and the *jordanon* but also some unit established on an experimental basis, e.g. on compatibility, while the smaller experimental units might be indistinguishable in the herbarium. If a complementary system were adopted, at least as a working hypothesis, the results of experimental taxonomic investigations could be co-ordinated with one another, and since the presentation of the facts could be so arranged as to show the synonymy, if any, between the previous taxonomic names and those of the experimentalist, it should not be difficult to compare the two systems or to employ experimental findings for the clarification of nomenclature if desired. A system has been suggested by Turesson (1922, pp. 344-5; 1929, p. 332; and 1930) which has already been employed in several investigations (Müntzing, 1930, p. 329; Gregor, 1931, p. 212; and Winge, 1933), and which will be adopted in the present studies. Its units are

the *coenospecies*, which has some affinity to Linnaeus's conception of a species, the *ecospecies* and the *ecotype*, and these have their equivalents in the artificially bred economic plants.

The present writers interpret Turesson's classificatory units as follows:

Coenospecies. A group distinguished by morphological, physiological or cytological characters, or a combination of these; separated from all other plants by sterility or by failure of hybrids to produce viable seed. Parts of a coenospecies may have become separated by natural barriers, e.g. oceans or mountain ranges, so that all potential hybridizations cannot occur in Nature.

Ecospecies. A group also distinguished by morphological, physiological or cytological characters, or a combination thereof; separated from other parts of its coenospecies by restricted interfertility or by failure of hybrids to establish themselves in Nature.

Ecotype. A population distinguished by morphological and physiological characters, most frequently of a quantitative nature; interfertile with other ecotypes of the ecospecies, but prevented from freely exchanging genes by ecological barriers. Spatially widely separated ecotypes may exhibit characters determined by genes restricted to the geographical regions in which they occur.

It may be thought that these definitions do nothing more than give yet another meaning to the species, variety and form units of common usage. It seems to the authors, however, that as the categories of experimental taxonomy are to be based on new criteria these should be at least provisionally indicated by the use of appropriate units, rather than that an attempt should be made to modify the meaning of the existing terms to fit the needs of the experimentalist. To illustrate the difficulties arising out of an attempt to reconcile the new treatment with the old terminology three examples may be cited where cytological and ecological differences of importance are not accompanied by striking morphological differences. (1) The species *Penstemon neotericus* Keck maintains its identity on account of a cytological difference, although it apparently represents a combination of the characters which distinguish two other very closely related species; this difference is associated with an extension of the range of *P. neotericus* into an area unoccupied by either of its two near relatives (Clausen, 1932). (2) *Phleum pratense* L. comprises diploid and hexaploid groups which are practically intersterile and occupy ecologically different habitats (Gregor, 1931). These two groups may correspond to var. *typicum* Beck. and var. *nodosum* (L.)

Richt. and together they probably fall within the sub-species *vulgare* A. & G. (3) In *Vaccinium uliginosum* L., also, polyploidy is followed by a distributional extension, but in this case the two groups have only the status of forms in taxonomic literature. "In general it may be said that the northern limit of the tetraploid form (*f. genuina* Herd.) lies slightly more to the north than the southern limit of the diploid form (*f. microphylla* Lange). Only in those regions (round the polar circle) can these two forms be found together, the diploid form being of a more markedly arctic distribution than the tetraploid" (Hagerup, 1933, p. 127). From these examples it can be seen that units of similar biological significance have been given the varying status of species, varieties and forms.

The principles of an experimental taxonomic investigation as at present conceived may be stated thus: (1) choice of a group of plants; (2) collection of samples over its geographical and ecological ranges; (3) cultivation of representative samples in an experimental garden; (4) observation of discontinuous, and biometrical comparison of continuous variations; (5) study of fertility relationships; (6) investigation of cytology; and (7) synthesis of results. In the present paper the first four operations are dealt with. Although the experiments described are mainly exploratory and have special reference to the sea plantains, the practical methods devised should be of use in other studies along similar lines. One of the fundamental features of this experimental attack is the substitution for the natural habitat, with its all too frequent irregularities, both physical and biotic, of an artificial and, as far as possible, a controlled environment in the form of a specially prepared experimental garden, where the polymorphy and characters of races can be studied under uniform conditions, and statistical methods applied. Character differences which have been accurately evaluated under these circumstances should reflect true hereditary dissimilarity. At the same time the experimentalist has the opportunity of thoroughly examining racial fertility relationships. The determination of the restrictions imposed upon the free diffusion of genes in Nature by sterility and other factors is a matter of very considerable taxonomic importance. Moreover, as sterility relationships have a bearing on genetical relationships, the simultaneous investigation of both is advantageous (for a discussion of these see Müntzing, 1930, pp. 314-30, and for genetical analytical methods see Matsuura, 1935, p. 145).

Experimental taxonomy should seek, therefore, to develop a method of classification which would take into consideration not only

the magnitude of the morphological and physiological differences of its units, but also the causes which maintain their identity, e.g. isolation in its various forms. But classificatory categories based primarily on such restrictions to gene exchange will, as in systems where other criteria are employed, inevitably lose their significant status if the dynamic nature of their components is not fully appreciated. Furthermore, if the system is to present successfully the results of experimental investigation, it will require to be sufficiently flexible to meet the needs of investigators working with divers kinds of material, and to provide a framework for future taxonomic development.

II. METHODS OF SAMPLING POPULATIONS

Material

The investigations in progress at this institute embrace an experimental study of the sea plantains (*Plantago maritima* L. and allied species) of Europe and North America. The present paper, the first of a series, deals with methods of sampling habitat populations, and their examination under experimental conditions. In succeeding papers it is hoped to deal with such aspects as character correlations, the races occurring in Britain, and the interrelationships of the North European and North American sea plantains.

This plant was chosen for the investigations because of its suitability as experimental material, since its various characters lend themselves comparatively readily to statistical measurements. The populations studied were characterized, not so much by their possessing peculiar constituents, as by the differing proportions in which the various types occurred. It was probably the size characters and those characters which together make up growth habit which most obviously distinguished one population from another. Size, however, while ecologically important, is one of the many quantitative characters which does not lend itself to verbal treatment, and which, moreover, cannot be studied with accuracy in the wild.

Collection of material

In preliminary experiments, plants were transferred from the natural habitats to the garden, but for several reasons, e.g. the difficulty of distinguishing age groups in the wild and of transporting plants alive over long distances, this method was discontinued in favour of the taking of seed samples. In collecting seed samples, ripe spikes were systematically gathered from comparatively well-defined

but not necessarily spatially isolated habitats, the number of spikes per plant collected from any one habitat depending on the density of the plantain population.

Cultivation of material

Each seed sample was sown in a heated glasshouse (60° F.), in a pan containing sterilized weed-free soil, during late February, the seedlings being later transferred to boxes placed in a cool house. In June they were transplanted to their positions in the experimental garden. The garden (Fig. 1) was planned so that each year one-third

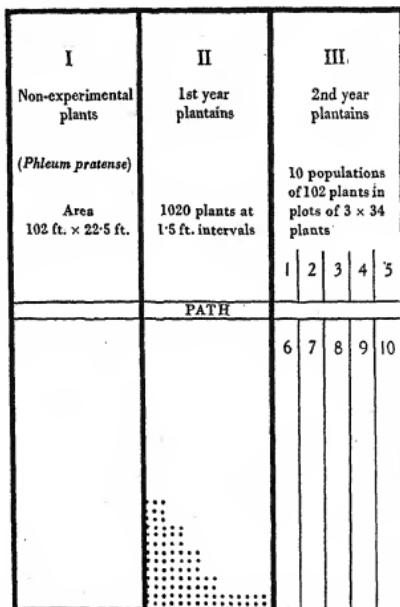


Fig. 1. Experimental garden.

of its area contained one-year-old plantains, one-third two-year-old plantains, and one-third a non-experimental crop. Ten populations could be examined each year, either systematically arranged as plots of 102 plants in three rows of thirty-four, or as six rows of seventeen plants distributed at random. The history of each section was as follows. The young plantains planted out in June were generally not observed during the first summer. They survived the winter and

became the experimental material of the second summer, at the end of which they were discarded and *carted off*. A definite amount of well-rotted farmyard manure was then applied to the vacated section and in the spring *Phleum* plants were grown; in the autumn these also were carted off. By this treatment about 18 months elapsed between the removal of one crop of plantains and the introduction of the next, thus giving time for cleaning and for the manure to mix thoroughly with the soil.

Characters and statistics

Every plant received a number and the data relating to each were systematically recorded. The samples for laboratory examination were collected in numbered specimen tubes contained in a carrier. A description of the characters studied is given below. Owing to limitations of space, it has been found necessary in the tables to utilize abbreviations of which the following is a list:

A, anther.	H, height.	Sc, scape.
AT, anther tip.	HbG, habit grade.	Sd, seed.
B, breadth.	Ix, index.	Sep, sepal.
Br, bract.	L, length.	Sp, spike.
D, density.	Lf, leaf.	Th, thickness.
F1G, flowering grade.	S, spread.	

Examples: LfL=leaf length, AL=anther length, BrB=bract breadth, etc.

Size of organs.

LfL and LfB (mature characters). The largest leaf on each plant is chosen by eye (28 July). Its length is measured in cm. and its maximum breadth in mm.

ScL and SpL (mature characters). The longest scape on each plant is chosen by eye and is pulled, not cut (25 July). Scape length includes rachis, spike length extends from the lowest floret to the apex; both are measured in cm.

LfTh and ScTh. Measured correct to 0.1 mm. with a micrometer screw gauge. Leaf thickness is taken at the midpoint on the midrib of the longest leaf (5 May). Scape thickness at the midpoint of the longest scape (25 July).

The following characters are measured by arranging the organs in rows on a slide smeared with glycerine and using a microscope with

a movable stage. The micrometer units, each of which is equivalent to 0.04286 mm., have been retained in this paper.

BrL, BrB, SepL and SepB. A mature spike, not one of the earliest to flower, is chosen, and from the midpoint a bract is removed. The abaxial sepal underlying this bract and to its left relative to the dorsal aspect is also detached, and both are placed, convex side uppermost, on the slide. Lengths are measured from apex to base; breadths across the widest point, that of the bract including the membranous margins.

AL and ATL. An anther is detached from a flower at the mid-point of a spike after exertion and before dehiscence, and placed with filament uppermost on the slide. Anther length is the total length including subulate tip, anther tip length is the length of the tip from apex to the level of the anther lobes.

SdL and SdB. From a ripe capsule containing two well-developed seeds the larger is chosen. This is placed on the slide convex surface uppermost. Both length, and breadth at the widest point, are measured exclusive of membranous margins.

Size of plants.

LfS, LfH, ScS and ScH. Spread is taken as the distance in inches between the apices of the longest leaves, or longest scapes, at opposite points in the perimeter of the plant. Height is measured from ground-level to the highest point of the leaf or scape system, half inches being the grouping units for leaf, and inches for scape. The leaves are measured prior to exertion of spikes (5 May), the scapes at maturity (14 July).

Arrangement of flowers.

SpD. A numerical expression of the density of spike. The number of flowers, or parts thereof, attached to the middle cm. cut with a razor from a spike which is mature but not one of the earliest.

Indices and other ratios.

BrIx, SepIx and SdIx. These three ratios, which indicate the shapes of the respective organs, are calculated for each plant from the data of length and breadth.

BrL : SepL, ScL : SpL, ScL : LfL. These ratios are calculated for each plant from the specified measurements. They represent certain



Fig. 2.

relationships between size of parts which have been employed in taxonomic description.

Estimates of habit and time of flowering.

LfS : H and ScS : H. Ratios indicating growth habits of leaf and scape systems.

HbG. An arbitrary estimate of habit, giving somewhat more information than the scape ratio. It consists of five grades ranging from decumbent to erect. In grade I spikes are restricted to the periphery, in the other four they are evenly distributed.



Fig. 3.

This "character" is dependent on the observer, though controlled by reference to standard photographs (Gregor, 1930, Pl. I and II). Owing to the small number of classes, it is the least satisfactory for statistical studies.

FIG. In order to obtain statistical data of time of flowering, each plant is classed as being at one of the following eleven stages:

- (1) Spikes not yet visible.
- (2) Spikes just visible.
- (3) Spikes easily visible.
- (4) Spikes well developed.
- (5) Stigmas of lower flowers visible.
- (6) Plants beginning to flower; up to approximately 12 per cent of spikes flowering.
- (7) Plants flowering sparsely; approximately 25 per cent of spikes flowering.
- (8) Plants flowering freely; approximately 50 per cent of spikes flowering.
- (9) Plants flowering abundantly; approximately 75 per cent of spikes flowering.
- (10) Spikes fading; approximately 12 per cent of spikes completed flowering.
- (11) Spikes much faded; approximately 25 per cent of spikes completed flowering.

Such a classification is, of course, dependent on the judgement of the observer, but the grades provide a relative order of flowering, the higher variates being the earlier. Observations are made on three

occasions, 1, 8 and 28 June. The first and last generally show skew distributions, while those of the middle date are more symmetric and afford the best picture of relative maturity.

Although some of the size characters examined showed curves with somewhat skew tails in the higher range they all seemed sufficiently unimodal to receive normal statistics. In fact the majority of the characters studied, including all those defined above, proved amenable to statistical treatment. In the cases where such methods were rejected it was on account of unsuitable frequency distributions.

For describing populations two main statistics are used, one to give the average of values, the *mean*, M ; and the other to show their variability, the *coefficient of variation*, C , which is merely an expression in percentage form of the *standard deviation*, σ . The accuracy of these is indicated as \pm their *standard errors*; for the mean this is σ/\sqrt{n} , where n is the number of observations (Fisher, 1932, p. 48); while for C it can be found from Pearson (1914, p. xxii). The *significance* of a difference between a pair of M or C values can be expressed as D/E_d , or the ratio of *difference: error of difference* (where $E_d = \sqrt{E_1^2 + E_2^2}$); the odds against D/E_d exceeding 2.58 by accident are 100 : 1, so that > 3 can be taken as a convenient measure of significance. Two types of correlation are used for special purposes in Tables III and IV, both having the coefficient r (see Fisher, 1932, *interclass* Chap. vi, *intraclass* p. 193). The methods applicable to uniformity trials and for dealing with populations in randomized plots, in so far as they have reference to the present paper, may be found in Fisher (1932, pp. 242 *et seq.*), or in Fisher & Wishart (1930).

III. RELIABILITY OF SAMPLING METHODS

Single plant sampling

The experiments of which the results are summarized in Tables I and II were designed to ascertain the validity of the methods of sampling described above for the various characters. In order to find out how the variability within a single plant compares with that of the whole population, the entire complement of certain organs of a few individual plants was measured. From Table I it will be seen that the variability of these organs equals or even exceeds that of the whole population; which indicates that reliable data concerning populations cannot be obtained by random sampling of their constituent plants. Table II shows that even parts on the same organ, e.g. bracts and sepals on the same spike, have a variability compar-

Experimental Taxonomy

TABLE I. Coefficients of variation for individual plants and parent populations

Character	Population PMN 44				Population PMN 54 (a)				Population PMN 81				Population PMN 84			
	Plant A	Population	Plant B	Population	Plant	Population	Plant	Population	Plant	Population	Plant	Population	Plant	Population	Plant	Population
ScL	11.59 ± .684	12.96 ± .933	10.80 ± .474	13.91 ± .778	11.74 ± .828	6.81 ± .477	13.45 ± 1.294	8.82 ± .478	14.37 ± 1.043	8.82 ± .478	14.37 ± 1.043	8.82 ± .478	14.37 ± 1.043	8.82 ± .478	14.37 ± 1.043	
SpL	23.31 ± 1.442	21.41 ± 1.582	24.30 ± 1.113	33.70 ± 2.048	17.42 ± 1.268	15.60 ± 1.118	17.58 ± 1.714	22.85 ± 1.392	19.79 ± 1.420	22.85 ± 1.392	19.79 ± 1.420	22.85 ± 1.392	19.79 ± 1.420	22.85 ± 1.392	19.79 ± 1.420	
Brl	13.40 ± .801	11.62 ± .837	12.30 ± .832	12.38 ± .884	10.94 ± .775	7.33 ± .788	10.68 ± .552	10.79 ± .552	10.79 ± .552	10.68 ± .552	10.79 ± .552	10.68 ± .552	10.79 ± .552	10.68 ± .552	10.79 ± .552	
BrB	13.44 ± .860	11.91 ± .858	14.53 ± .643	15.32 ± .860	9.54 ± .672	9.93 ± .632	8.85 ± .836	10.74 ± .589	8.90 ± .646	8.85 ± .836	10.74 ± .589	8.90 ± .646	8.85 ± .836	10.74 ± .589	8.90 ± .646	
Brix	6.77 ± .394	13.70 ± .992	6.73 ± .993	15.92 ± .896	9.60 ± .620	7.77 ± .474	7.58 ± .716	6.62 ± .339	7.74 ± .562	7.58 ± .716	7.74 ± .562	7.58 ± .716	7.74 ± .562	7.58 ± .716	7.74 ± .562	
SepL	5.93 ± .348	7.34 ± .521	4.64 ± .201	10.88 ± .564	6.77 ± .476	6.21 ± .435	5.84 ± .552	6.21 ± .435	6.21 ± .435	5.84 ± .552	6.21 ± .435	5.84 ± .552	6.21 ± .435	5.84 ± .552	6.21 ± .435	
Sepl	5.62 ± .368	10.26 ± .737	8.35 ± .371	7.70 ± .443	7.77 ± .547	5.37 ± .376	6.21 ± .435	5.37 ± .376	6.21 ± .435	5.37 ± .376	6.21 ± .435	5.37 ± .376	6.21 ± .435	5.37 ± .376	6.21 ± .435	
SeplX	7.22 ± .424	11.42 ± .822	7.20 ± .331	8.66 ± .609	5.67 ± .404	7.87 ± .744	7.69 ± .447	10.42 ± .765	7.69 ± .447	7.87 ± .744	7.69 ± .447	7.87 ± .744	7.69 ± .447	7.87 ± .744	7.69 ± .447	
BrL : SepL	11.08 ± .558	10.53 ± .757	9.75 ± .423	20.74 ± 1.186	9.85 ± .693	7.02 ± .491	8.90 ± .842	7.44 ± .403	7.97 ± .578	8.90 ± .842	7.44 ± .403	7.97 ± .578	8.90 ± .842	7.44 ± .403	7.97 ± .578	
Mean	10.98	12.35	10.96	17.12	10.40	8.17	9.40	10.10	11.08	9.40	10.10	9.40	10.10	9.40	10.10	

TABLE II. Data from individual spikes of PMN 54 plant (27)

Character	Entire wing space						Coefficients of variation (%)
	Lower 1/3	Middle 1/3	Upper 1/3	Lower spike Mean values	Middle 1/3	Upper 1/3	
BrL	104.37 ± .870	88.08 ± 1.406	63.66 ± .555	85.08 ± 1.522	81.84 ± .551	73.68 ± .481	61.05 ± .823
BrB	44.72 ± .495	39.87 ± .268	37.14 ± .340	40.53 ± .341	38.64 ± .422	39.83 ± .250	35.00 ± .403
SepL	69.93 ± .213	63.34 ± .198	63.37 ± .230	67.17 ± .247	65.66 ± .191	61.87 ± .249	56.87 ± .257
SepB	35.64 ± .200	36.57 ± .283	34.19 ± .005	35.43 ± .177	34.42 ± .228	34.00 ± .199	34.99 ± .235
Brl: SepL	1.40 ± .014	1.29 ± .019	1.73 ± .016	1.26 ± .019	1.22 ± .010	1.22 ± .007	1.11 ± .010
Brlx	2.35 ± .026	2.21 ± .030	1.86 ± .012	2.09 ± .027	2.13 ± .021	2.00 ± .019	1.96 ± .017
Sepix	1.97 ± .020	1.87 ± .017	1.86 ± .012	1.90 ± .010	1.89 ± .014	1.91 ± .012	1.88 ± .007

able with that of the whole plant or even of the population. It is shown, moreover, that the variability of these parts is greater on the earliest spikes than on later ones, and that the region of least variability is the middle third of the later flowering spikes (cf. p. 330). It might be thought that the best method of estimating the mean value for a character throughout a population would be to calculate the mean of each plant for that character, as in Table I, or at least to base this calculation on as large a sample from each plant as possible. There are, however, certain obvious practical objections to such a method, for it would be extremely laborious and might seriously damage the plants. Apart, however, from these objections the method itself is open to criticism. For example, two equally long-leaved plants might easily be found to have different mean values for leaf length if one of them possessed a greater proportion of immature leaves at the time of examination.

It will be seen, then, that for the examination of populations comprising many plants it is a practical necessity to reduce the number of samples per plant to an absolute minimum, that is to one, if it can be ascertained that such a procedure is consistent with accuracy. It is necessary, therefore, to substitute *selective* for *random* sampling in order to obtain specimens that may be regarded as equivalent to one another. Pearson (1901) showed that by selecting organs of like maturity and similar position on the plant he obtained comparable specimens upon which to measure characters. These he termed *homotypes*, and he showed, by means of intraclass correlations on vast quantities of material, that homotypes within a plant are strongly correlated, or much more alike than are homotypes of different plants of a habitat population. Some small tests of this nature have been made on the validity of certain characters, advantage being taken of Harris's short method of calculation (Fisher, 1932, p. 193). It facilitates the work of recording if several characters can be measured on a selected organ. For example the longest leaf and scape, which are homotypes readily distinguishable by eye, are chosen for measurements of length, breadth and thickness; while for bract and sepal measurements a floret is taken from the midpoint of a mature and somewhat late flowering spike, thus limiting the sampling to the region of least variability on a spike with comparatively low variability (see Table II). Selective methods for other characters have been noted in the descriptions (pp. 329-30).

From each of 102 plants, quintuplicate selections were made, i.e. the five longest leaves and scapes, and five spikes for measurements of

sepals, bracts and spike density. Ten anthers were taken from each of fifty-four plants since there was difficulty in finding 102 plants in flower at the same time. In Table III the likeness of characters may be most readily seen from the intraclass correlation coefficients, where $+1.0$ would imply that they were identical. An alternative method of expression is the comparison of "variances" ($=\sigma^2$), upon which Fisher's z test of significance can be applied. It will be seen

TABLE III. *Intraclass correlation to test validity of homotypes*

PMN 44. 102 plants, 5 homotypes. Anthers 54 plants, 10 homotypes.

Character	Coefficient of correlation	Variance		$\frac{z}{(n_1=10)} \times 100$	$\frac{s.e.}{M} \times 100$
		Between plants	Within plants		
LfL (5 May)	+ .959	78.91	0.623	2.42	0.404
LfB (5 May)	+ .738	12.36	0.802	1.37	1.323
LfTh (5 May)	+ .657	0.42	0.0039	1.19	0.656
LfL (28 July)	+ .930	93.17	1.319	2.13	0.408
LfB (28 July)	+ .919	19.60	0.326	2.05	0.786
SpL	+ .729	24.90	1.691	1.35	1.305
ScL	+ .948	161.50	1.645	2.29	0.318
ScTh	+ .930	0.341	0.0048	2.13	0.380
BrL	+ .853	52.23	1.793	1.71	0.587
BrB	+ .746	49.06	3.064	1.39	0.606
BrIx	+ .847	0.396	0.0120	1.69	0.473
SepL	+ .934	13.96	0.186	2.16	0.228
SepB	+ .685	27.97	2.319	1.25	0.598
SepIx	+ .688	0.278	0.0227	1.25	0.671
SpD	+ .737	22.13	1.446	1.36	0.914
		$(n_1 = 53)$ $(n_2 = 486)$			
AL	+ .856	196.98	3.158	2.07	0.350
ATL	+ .828	20.57	0.405	1.96	0.970

that the correlations range from $+0.96$ to $+0.66$, and that there is no question as to their significance. As length is the criterion in choosing scape and leaf it is gratifying to find high uniformity among the five longest. Other characters measured on these organs show more variability, especially leaf thickness. Variance within plants is now much smaller than between plants (cf. Table I), while the standard error for 100 observations, expressed as a percentage of their mean, affords some idea of the extent of errors due to sampling a population, for it exceeds 1 per cent in only two cases.

When some studies (unpublished) were being made on the ordinary correlations between characters (interclass), a special question of sampling arose, as to what difference there might be in using pairs of measurements made on one organ as opposed to pairs on two similar organs of a plant. Pearson (1901) has explored this question and shown that interclass correlations of characters on the

same organ, which he called *organic*, gave somewhat higher values than those on different organs (*cross-homotypic*). Interclass correlations of these kinds have been calculated to ascertain to what extent such discrepancies might affect the plantain studies. Employing data gathered for Table III, records of the characters of four homotypes from each of 102 plants afforded 408 pairs for correlation, while in the case of anthers, ten homotypes on fifty-four plants gave 540 pairs. Correlating length with breadth of leaf for example, the *organic* coefficient was calculated from 408 pairs of measurements, each pair on a single leaf; while to obtain the *cross-homotypic* coefficient the length of leaf 1 was coupled with the breadth of leaf 2, and vice versa, the same being done with leaves 3 and 4 in each plant, so that 408 pairs were again utilized. In Table IV it will be seen that the organic coefficients (below and to the left of the diagonal) are somewhat

TABLE IV. Comparison of organic with
cross-homotypic correlations (PMN 44)

	Cross homotypic			Cross-homotypic			
	LfL	LfB	LfTh	SpL	Scl	ScTh	
5 May	—	+·444	+·201	SpL	—	+·725	+·678
LfL	+·456	—	+·197	ScL	+·734	—	+·587
LfB	+·218	+·196	—	ScTh	+·719	+·596	—
Organic			Organic				
28 July	LfL	{ Organic	+·252	AL	{ Organic	—·132	
	LfB	{ Cross-homotypic	+·242	ATL	{ Cross-homotypic	—·150	
Character	Cross-homotypic						
	BrL	BrB	BrIx	SepL	SepB	SepIx	SpD
BrL	—	+·523	+·577	+·620	+·270	+·226	—·232
BrB	+·628	—	—·072	+·439	+·427	—·064	—·283
BrIx	+·649	+·167	—	+·360	—·071	+·347	—·029
SepL	+·655	+·463	+·383	—	+·315	+·387	—·261
SepB	+·304	+·488	+·094	+·318	—	+·469	—·113
SepIx	+·228	+·096	+·388	+·442	+·671	—	+·089
SpD	+·286	+·331	+·020	+·265	+·169	+·043	—
Organic							

greater than the cross-homotypic values (above and to the right). This applies to both positive and negative correlations, with three or four exceptions in all of which the coefficients are insignificant. The magnitude of differences is generally small, averaging about 0·03, but occasionally exceeding 0·1, and it seems to depend on the strength of correlation in conjunction with variability of the homotypes within the plant (contrast behaviour in scape and bract groups). It was decided that organic characters should be used for the studies in question, because there is less likelihood of errors due to sampling.

To avoid confusion a brief restatement may be made: (1) characters on different organs of a plant, e.g. on the leaves, vary enormously; (2) random sampling would give a very imperfect idea of the plant; (3) some form of selective sampling is needed, (4) this is achieved by selecting organs that are apparently equivalent (homotypic), such as the longest leaf, scape, etc.; (5) a statistical test (Table III) shows that the methods of sampling appear to be justified, although all characters are not equally satisfactory; (6) ratios such as bract length : breadth should naturally be measured on the same organs, and when correlation studies are contemplated it seems advisable (Table IV) to measure as many characters as possible on the same organ.

Influence of season on population data

(a) *Within years.* The examination of replicate samples of the same population in the same season afforded a guide to the reliability attainable by the adopted sampling technique. In Table V (A and B) are shown the mean values for various characters for duplicate samples of populations PMN 18 and PMN 20 examined in the same year. With the exception of the values for leaf and scape thickness in PMN 18, no mean difference exceeds twice its standard error, showing that such differences as do occur can safely be regarded as insignificant. An estimate of the magnitude of the mean differences, in percentage form, is given for comparative purposes. In Table V (C) two closely related populations PMN 21 and PMN 44 are compared. These were collected from the same habitat in the same year, but by different methods. PMN 21 was derived from a seed sample collected in the manner already described (p. 327), while PMN 44 was raised from a sample of plants transferred from the wild and seeded in isolation at Edinburgh. As in the duplicated samples of PMN 18 and PMN 20 the differences between the character mean values fail to reach the significant level. In contrast with the above the mean values for two different habitat populations (Table V (D)) exhibit differences which are unquestionably significant, that of the scape height means being as great as 44·8 times its error. These data show that when populations are compared in the same year and at the same place the accuracy of the technique is sufficient to ensure the detection of real differences in population characteristics.

In 1930 samples of two populations, PMN 21 and PMN 32, were grown from seed at Edinburgh. One 192-plant sample of each was transplanted into the experimental garden at this institute on 30 June,

and duplicate samples were sent to Dr W. B. Turrill at Kew, Surrey, and transplanted into the garden there on 3 July. Records of time of flowering and plant height were made in the following season

TABLE V. Reliability of within-year data from 100-plant plots

Character	A. Duplicate plots of PMN 18				B. Duplicate plots of PMN 20			
	(a) <i>M</i>	(b) <i>M</i>	$D \times 100$ <i>M</i>	D <i>E_d</i>	(a) <i>M</i>	(b) <i>M</i>	$D \times 100$ <i>M</i>	D <i>E_d</i>
LfL	27.95 ± .492	27.35 ± .551	2.17	0.81	22.66 ± .500	20.94 ± .474	6.11	1.92
LfB	6.77 ± .183	7.01 ± .169	3.48	0.96	5.44 ± .162	5.48 ± .168	0.73	0.17
LfTh	1.01 ± .016	1.11 ± .017	9.43	4.22	1.18 ± .017	1.19 ± .016	0.84	0.43
ScL	36.30 ± .552	36.40 ± .493	0.28	0.14	33.04 ± .723	31.65 ± .619	4.30	1.46
ScTh	1.94 ± .023	1.81 ± .022	6.93	4.10	2.10 ± .027	2.05 ± .027	2.41	1.32
SpL	9.75 ± .182	9.71 ± .163	0.41	0.17	10.06 ± .218	10.14 ± .243	0.79	0.25
LfS	9.14 ± .234	9.58 ± .214	4.70	1.39	10.10 ± .249	9.44 ± .247	6.76	1.88
LfH	3.59 ± .121	3.75 ± .106	4.36	0.99	2.85 ± .083	2.62 ± .086	8.41	1.93
ScS	18.20 ± .330	17.61 ± .309	3.30	1.32	17.02 ± .274	16.37 ± .286	3.89	1.64
ScH	14.26 ± .234	14.43 ± .243	1.19	0.48	11.62 ± .300	11.15 ± .300	4.13	1.11
LfS : H	2.62 ± .067	2.58 ± .053	1.54	0.47	3.62 ± .165	3.61 ± .170	0.28	0.04
ScS : H	1.27 ± .023	1.25 ± .020	1.59	0.66	1.54 ± .032	1.57 ± .047	1.45	0.52
C. Related populations								
PMN 21	PMN 44	$D \times 100$	D	PMN 32	PMN 42	$D \times 100$	D	
<i>M</i>	<i>M</i>	<i>M</i>	<i>E_d</i>	<i>M</i>	<i>M</i>	<i>M</i>	<i>E_d</i>	
LfL	27.21 ± .530	27.28 ± .418	0.26	0.10	15.82 ± .522	36.01 ± .678	77.91	23.60
LfB	6.02 ± .154	6.06 ± .136	0.66	0.20	4.14 ± .181	10.83 ± .274	89.38	26.64
LfTh	1.08 ± .015	1.10 ± .014	1.83	0.99	1.02 ± .018	1.24 ± .021	19.47	8.04
ScL	38.59 ± .584	38.49 ± .422	0.26	0.14	22.09 ± .538	51.93 ± .595	80.63	37.21
ScTh	1.70 ± .027	1.78 ± .024	4.60	2.23	1.72 ± .029	2.01 ± .022	15.55	8.03
SpL	9.15 ± .239	9.78 ± .175	6.66	2.25	6.62 ± .181	9.46 ± .196	35.32	10.64
LfS	13.89 ± .291	14.51 ± .238	3.72	1.38	6.08 ± .219	18.21 ± .271	99.88	34.80
LfH	4.27 ± .121	4.47 ± .112	4.58	1.23	1.81 ± .073	7.41 ± .151	121.48	33.45
ScS	19.45 ± .377	19.80 ± .258	1.78	0.77	13.06 ± .312	20.04 ± .396	42.18	13.84
ScH	15.39 ± .270	15.60 ± .183	1.36	0.61	7.06 ± .227	22.40 ± .257	104.14	44.79
LfS : H	3.42 ± .083	3.42 ± .070	0.00	0.00	3.51 ± .098	2.52 ± .043	32.84	9.26
ScS : H	1.29 ± .030	1.29 ± .017	0.00	0.00	1.98 ± .056	0.90 ± .013	75.00	18.80

simultaneously at each centre. The results are given in Table VI. The order both of flowering and height of plants remained unchanged at both localities, but the late-flowering low-growing population, PMN 32, responded more to the changed environment than did PMN 21.

TABLE VI. Mean values for flowering grade and scape height at two centres

Population	PMN 21		PMN 32	
	FIG	ScH	FIG	ScH
Character				
Edinburgh	3.49 ± .132	15.39 ± .270	2.43 ± .146	7.06 ± .227
Kew	2.95 ± .141	15.72 ± .221	1.29 ± .075	9.30 ± .281
$M' - M''$				
$\frac{M'}{M} \times 100$	16.77	2.12	61.29	27.38
Difference	2.798	0.946	6.960	6.202
Error of D				

The populations, however, tended to be later flowering and larger at Kew, significantly so in the case of PMN 32. The coefficients of variation, nevertheless, remained similar in both environments, e.g., for plant height the coefficients of variation for PMN 21 were 17.73 per cent \pm 1.280 at Edinburgh and 14.01 per cent \pm 0.964 at Kew, and the corresponding values for PMN 32 were 32.25 per cent \pm 2.482 and 30.21 per cent \pm 3.325.

(b) *Between years.* When samples of the same population were examined in four different years, the differences between the extreme yearly means of the majority of characters exceeded three times their respective errors (Table VII); the corresponding coefficients of varia-

TABLE VII. *Reliability of mean values of different years (PMN 44)*

Character	Mean values				General mean	Greatest diff. \times r _{oo}	Gen. mean	Greatest diff.	Error of D
	1931	1933	1934	1935					
LfL	27.28 \pm .418	27.71 \pm .440	27.50 \pm .462	26.80 \pm .409	27.32	3.33	1.52		
LB1	6.06 \pm .136	7.02 \pm .176	7.00 \pm .166	7.32 \pm .171	6.85	1.83 ³⁹	5.76		
LfTh	1.10 \pm .014	0.87 \pm .016	0.79 \pm .012	0.94 \pm .011	0.93	33.33	17.08		
ScL	38.49 \pm .422	41.15 \pm .464	40.70 \pm .527	41.57 \pm .498	40.48	7.61	4.72		
ScTh	1.78 \pm .024	1.60 \pm .024	1.63 \pm .023	1.87 \pm .021	1.72	15.70	8.51		
SpL	9.78 \pm .175	9.99 \pm .202	9.80 \pm .210	10.22 \pm .182	9.95	4.52	1.74		
SpD	13.36 \pm .177	13.65 \pm .219	13.12 \pm .197	12.91 \pm .225	13.26	5.58	2.36		
BrL	64.33 \pm .910	60.65 \pm .862	65.00 \pm .759	67.00 \pm .983	64.25	9.88	4.86		
BrB	29.07 \pm .313	26.80 \pm .321	29.00 \pm .347	29.07 \pm .367	28.49	7.97	4.66		
BrIx	2.25 \pm .030	2.27 \pm .028	2.26 \pm .031	2.31 \pm .029	2.27	2.64	1.44		
SepL	—	57.20 \pm .558	57.33 \pm .423	56.59 \pm .503	57.04	1.30	1.13		
SepB	—	24.64 \pm .274	24.86 \pm .256	25.60 \pm .245	25.03	3.84	2.61		
SepIx	—	2.35 \pm .028	2.32 \pm .027	2.23 \pm .026	2.30	5.22	3.13		
SdL	53.30 \pm .352	57.28 \pm .369	55.91 \pm .399	57.29 \pm .363	55.95	7.13	7.89		
SdB	23.13 \pm .135	23.82 \pm .129	23.88 \pm .143	23.46 \pm .171	23.62	3.18	3.82		
SdIx	2.30 \pm .014	2.42 \pm .018	2.35 \pm .016	2.43 \pm .017	2.38	5.46	5.93		
AL	47.30 \pm .627	44.60 \pm .391	46.39 \pm .361	46.08 \pm .367	46.09	5.86	3.66		
ATL	6.76 \pm .211	6.59 \pm .123	6.23 \pm .097	6.34 \pm .114	6.48	8.18	2.29		
FIG	3.86 \pm .117	5.06 \pm .154	3.98 \pm .137	3.73 \pm .106	4.16	31.97	7.11		
LFS	14.51 \pm .238	16.11 \pm .279	14.26 \pm .237	15.42 \pm .278	15.08	12.28	5.49		
LfH	4.47 \pm .112	4.46 \pm .105	5.41 \pm .124	4.89 \pm .128	4.81	19.71	5.84		
ScS	19.80 \pm .258	20.67 \pm .269	21.33 \pm .274	19.22 \pm .263	20.25	10.41	5.10		
ScH	15.60 \pm .183	16.50 \pm .189	16.62 \pm .241	16.62 \pm .220	16.36	6.23	3.37		
LfS : H	3.42 \pm .070	3.80 \pm .097	2.74 \pm .062	3.30 \pm .074	3.31	32.02	9.21		
ScS : H	1.29 \pm .017	1.26 \pm .018	1.31 \pm .019	1.18 \pm .017	1.26	10.32	5.04		
HbG	2.43 \pm .053	2.92 \pm .073	2.84 \pm .061	2.89 \pm .064	2.77	17.69	5.91		
BrL : SepL	—	1.06 \pm .010	1.73 \pm .012	1.18 \pm .015	1.12	10.71	6.69		
ScL : SpL	—	4.25 \pm .072	4.30 \pm .072	4.18 \pm .067	4.24	2.83	1.22		
ScL : LfL	—	1.55 \pm .020	1.51 \pm .020	1.59 \pm .017	1.54	5.19	3.09		

tion on the other hand showed less fluctuation (Table VIII). From Table VII it will be seen that the leaf characters, with the exception of length, vary markedly from year to year. Leaf spread, height and

thickness, being immature characters, fluctuate according to whether spring growth is late or early; they naturally are liable to greater seasonal fluctuations than leaf length, which is a mature character.

TABLE VIII. *Reliability of coefficients of variation between years (PMN 44)*

Character	Coefficients of variation (%)				General mean	Greatest diff. $\times 100$	Gen. mean	Greatest diff.	Error of D.
	1931	1933	1934	1935					
LfL	17.87 ± 1.12	15.86 ± 1.15	16.79 ± 1.22	15.34 ± 1.11	16.47	15.36	1.604		
LfB	25.90 ± 1.68	25.08 ± 1.88	22.86 ± 1.70	23.55 ± 1.75	24.35	12.48	1.272		
LfTh	13.91 ± 0.86	18.45 ± 1.34	15.09 ± 1.09	12.22 ± 0.87	14.92	41.76	3.899		
ScL	12.87 ± 0.79	11.27 ± 0.82	12.96 ± 0.93	11.97 ± 0.86	12.27	13.77	1.371		
ScTh	15.55 ± 0.97	14.78 ± 1.07	14.36 ± 1.04	11.29 ± 0.81	14.00	30.43	3.370		
SpL	20.82 ± 1.32	20.25 ± 1.49	21.41 ± 1.18	17.80 ± 1.30	20.07	17.99	1.764		
SpD	13.28 ± 0.95	16.66 ± 1.17	14.98 ± 1.08	17.56 ± 1.27	15.47	27.67	2.699		
BrL	14.00 ± 1.00	14.20 ± 1.02	11.62 ± 0.84	14.82 ± 1.06	13.66	23.43	2.366		
BrB	10.79 ± 0.77	11.96 ± 0.86	11.91 ± 0.86	12.77 ± 0.91	11.86	16.69	1.661		
BrIx	13.04 ± 0.93	12.42 ± 0.89	13.70 ± 0.99	12.96 ± 0.90	13.03	9.90	0.969		
SepL	—	9.8 ± 0.69	7.34 ± 0.52	8.97 ± 0.63	8.71	28.36	2.859		
SepB	—	11.14 ± 0.79	10.46 ± 0.74	9.63 ± 0.68	10.35	14.40	1.429		
SepIx	—	11.94 ± 0.86	11.42 ± 0.82	11.86 ± 0.84	11.74	4.43	0.438		
SdL	9.33 ± 0.47	7.94 ± 0.56	7.14 ± 0.50	6.34 ± 0.45	7.69	38.88	4.595		
SdB	7.88 ± 0.39	6.52 ± 0.46	5.97 ± 0.42	7.24 ± 0.51	6.90	27.68	3.332		
SdIx	8.70 ± 0.43	7.42 ± 0.52	6.83 ± 0.48	6.90 ± 0.49	7.46	25.07	2.902		
AL	9.37 ± 0.94	8.83 ± 0.62	7.78 ± 0.55	8.00 ± 0.56	8.50	18.69	1.460		
ATL	21.97 ± 2.30	18.59 ± 1.36	15.56 ± 1.33	18.08 ± 1.31	18.54	34.25	2.478		
FIG	35.13 ± 2.38	30.79 ± 2.35	34.45 ± 2.71	28.66 ± 2.17	32.26	20.06	2.009		
LfS	19.77 ± 1.20	17.47 ± 1.26	16.57 ± 1.20	18.24 ± 1.32	17.86	14.56	1.532		
LfH	29.11 ± 1.19	23.83 ± 1.76	22.94 ± 1.71	26.45 ± 1.98	25.58	24.12	2.406		
ScS	15.20 ± 0.94	13.16 ± 0.94	12.84 ± 0.92	13.70 ± 0.99	13.73	17.19	1.795		
ScH	13.65 ± 0.84	11.52 ± 0.82	14.51 ± 1.05	13.23 ± 0.95	13.23	22.60	2.245		
LfS : H	24.73 ± 1.34	25.79 ± 1.92	22.57 ± 1.67	22.63 ± 1.67	23.78	13.54	1.265		
ScS : H	15.31 ± 0.95	14.42 ± 1.03	14.86 ± 1.07	14.43 ± 1.04	14.76	6.03	0.635		
HbG	25.30 ± 1.63	25.22 ± 1.87	21.54 ± 1.59	22.25 ± 1.64	23.83	15.78	1.651		
BrL : SepL	—	9.79 ± 0.56	10.53 ± 0.76	12.53 ± 0.89	10.94	24.86	2.415		
ScL : SpL	—	16.96 ± 1.23	12.45 ± 0.95	16.11 ± 1.17	15.17	29.73	2.902		
ScL : LfL	—	13.36 ± 0.96	13.05 ± 0.94	10.67 ± 0.76	12.36	21.76	2.197		

In Table IX the mean values for the leaf characters, together with the differences between them expressed as a percentage of the 2-year mean, are given for PMN 21 in the seasons 1931 and 1935. It will be observed that the percentages range from 0.1 to 19.6. It has already been shown (Table V (C)) that PMN 21 and PMN 44 are similar; this being so the seasonal behaviour of the one should run parallel to that of the other. When, therefore, the mean values of PMN 21 are adjusted on the basis of the seasonal behaviour of PMN 44, the percentage differences should be reduced if the actual values reflect environmental influences. The corrected values do indeed show reductions, as may be seen in the lower half of Table IX. We may take as an

TABLE IX. *Seasonal fluctuations in leaf characters*
(PMN 21; 1931 and 1935)

The actual mean values are shown, and also values corrected for seasonal effect on the basis of a control population.

Actual values	LfS	LfH	LfS : H	LfL	LfB	LfTh
M' (1931)	13.98	4.27	3.42	27.21	6.02	1.08
M'' (1935)	14.83	4.73	3.34	27.25	7.33	0.95
M (of both)	14.41	4.50	3.38	27.23	6.68	1.02
$M' - M''$						
$\frac{M'}{M} \times 100$	5.901	10.222	2.367	0.147	19.626	12.808
Corrected values						
M' (1931)	14.53	4.59	3.31	27.25	6.81	0.91
M'' (1935)	14.50	4.65	3.35	27.78	6.86	0.94
M (of both)	14.52	4.62	3.33	27.52	6.84	0.93
$M' - M''$						
$\frac{M'}{M} \times 100$	0.207	1.299	1.201	1.926	0.732	3.226

example the figures for leaf spread. The appropriate mean values for this measurement were:

	1931	1935	4-year mean
PMN 44 ...	14.51	15.42	15.08
PMN 21 ...	13.98	14.83	

Now if the PMN 21 values are multiplied by the factor 4-year mean \div yearly mean for PMN 44, we have $13.98 \times 1.0393 = 14.53$, and $14.83 \times 0.9779 = 14.50$. The percentage difference is thus reduced from 5.9 to 0.2.

Influence of age on population data

It was not found possible to obtain reliable estimates of the characters of the reproductive parts during the first year of growth on account of the failure of some plants to produce scapes until the second year; moreover, the first season leaf characters were not a guide to the second-year performance. The second-year leaf lengths have invariably been found to exceed the value for the previous season. Leaf breadth, however, proved to be a more erratic character, the first season's measurements sometimes exceeding those of the second season as is shown in Table X. The greater breadth of leaf in the first year was found to occur most commonly in populations producing comparatively few leaves at that stage. When measuring maximum size characters, the date of sampling was a matter of importance. It will be noted from Table X that by 8 July the leaf length of population PMN 54 had almost attained its maximum. In some populations, however, the end of July was the date of maximum leaf development.

TABLE X
Differences between one- and two-year-old populations

Population	Mean values				Coefficient of variation			
	Leaf length		Leaf breadth		Leaf length		Leaf breadth	
	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
PMN 21	24.20 ± .540	27.21 ± .530	7.73 ± .239	6.02 ± .154	22.32 ± 1.65	18.79 ± 1.56	30.90 ± 2.36	25.83 ± 1.92
PMN 44	24.21 ± .407	27.28 ± .418	7.87 ± .201	6.06 ± .136	19.59 ± 1.23	19.36 ± 1.22	29.62 ± 1.95	25.90 ± 1.68

Character	Mean values				Coefficient of variation			
	5 May		8 July		16 August		16 August	
	LfL	16.74 ± .496	19.91 ± .646	30.67 ± .643	30.93 ± 2.37	21.80 ± 1.60	21.13 ± 1.54	26.91 ± 1.94
LB		7.36 ± .247	29.91	11.31 ± .291	33.52 ± 2.61			

Effect of date of sampling on leaf length and leaf breadth values (PMN 54)

Habitat sampling

The reliability of the method of habitat sampling adopted was tested by examining collections from the same habitats made in different years. Two habitats, *A* and *B*, were sampled in the years 1928 and 1930 respectively, and in 1933 the same habitats were revisited and other two samples obtained. In 1934 these four samples were sown and transplanted into the garden, each in a randomized arrangement of six rows of seventeen plants. The populations were examined in 1935. The significance of the data, in the form of the ratio D/E_d , is presented in Table XI. It will be seen that when the characters of the duplicate samples *A'* and *A''* were compared, and similarly those of *B'* and *B''*, the mean differences were less than three times their errors. The samples may therefore be regarded as truly representative of their habitats. When, however, the collections from the two different habitats *A* and *B* were compared, the mean differ-

TABLE XI. *Reliability of habitat sampling*

Inter- and intra-habitat collection comparisons.

Character	Difference Error of \bar{D} of mean values			Difference Error of \bar{D} of C values		
	$A' - A''$	$A - B$	$B' - B''$	$A' - A''$	$A - B$	$B' - B''$
LfL	1·05	16·44	1·87	1·26	0·49	0·34
LfB	0·51	12·13	0·22	0·70	0·83	0·19
LfTh	1·64	11·07	1·27	0·27	0·15	1·24
ScL	1·20	12·55	0·35	0·33	1·43	1·02
ScTh	1·35	22·84	1·02	0·10	0·21	0·66
SpL	0·32	8·81	1·28	1·25	0·01	0·31
SpD	2·44	1·07	0·05	1·45	0·57	1·68
BrL	0·10	3·38	1·55	0·88	0·31	1·04
BrB	0·86	10·90	0·31	0·47	1·34	2·88
BrIx	0·57	4·98	1·34	0·32	0·80	0·56
SepL	0·50	2·33	0·70	0·28	0·57	0·29
SepB	0·20	15·23	0·78	1·67	2·18	0·31
SepIx	0·32	11·13	0·11	0·51	1·63	0·48
SdL	0·01	18·49	0·05	0·02	0·03	0·01
SdB	0·04	8·85	0·05	0·02	0·01	0·07
SdIx	0·03	9·66	0·08	0·06	0·00	0·04
AL	0·14	1·71	0·11	0·67	1·92	0·00
ATL	0·69	1·62	0·81	0·65	0·25	0·87
FIG	0·18	17·71	0·40	0·49	0·62	0·25
LfS	1·27	8·32	0·75	0·96	0·27	2·44
LfH	0·18	2·12	1·22	0·42	0·07	0·98
ScS	0·00	4·62	0·06	1·08	0·80	0·70
ScH	1·15	15·45	0·68	0·48	1·62	1·04
LIS : H	0·97	0·23	1·58	0·45	0·92	0·73
ScS : H	1·84	18·43	0·00	0·66	0·18	1·01
HbG	0·73	9·35	0·73	1·96	3·16	0·32
BrL : SepL	0·06	2·35	2·16	0·75	1·04	0·00
ScL : SpL	0·89	0·67	1·51	0·67	0·80	0·41
ScL : LfL	2·27	1·87	0·76	1·55	0·39	1·71

ences, especially for the size and habit of growth characters, were clearly significant, as can be seen from column 3 of the table. The significances of the differences between the coefficients of variation are given in the last three columns. The only difference which was greater than three times its error was that between habitats *A* and *B* for the character habit grade, the erect population from habitat *B* having the lower value.

Where seed was difficult to obtain owing to the effect on seed production of high winds, grazing animals, etc., random samples of 200 or more plants were taken and seeded in isolation at Edinburgh. As already explained PMN 44 was a sample obtained in this way from plants taken from the natural habitat of PMN 21 in order to test the validity of the method. The similarity between these two populations, as shown in Table V (C), proves that no significant discrepancy arises through adopting this method of sampling.

Cultural methods

It has already been shown that a population reacts significantly to seasonal differences and to differences of locality. In any one season, soil irregularities or gradients and cultural treatment will tend to invalidate the results unless precautions are taken to minimize their effects. In this connexion the arrangement of sample plots and the choice of the number of plants to represent a population are matters of importance.

In order to establish the most suitable plot arrangement, a trial area was planted with 408 plants of population PMN 44 in twelve rows of thirty-four plants. This allowed for arbitrary divisions into smaller plots of varying size and shape, ranging from halves, lengthwise or crosswise, to single cross rows of only twelve plants. The plant characters examined for the purposes of the test were those which reflected growth vigour, e.g. leaf and scape length. Each of the 408 plants received an identification number and was examined separately.

(a) *Variability within plots.* The coefficients of variation for leaf and scape length for some of the plot arrangements are given in Table XII. It will be seen that, although the values for the 204-plant plots approach more closely the values for the whole plot than do those of the 102-plant plots, yet when the lengthwise, 3 × 34, arrangement of the latter is examined, the greatest deviations from the whole plot value are found to be only 1.14 and 1.55 per cent for leaf length in the first and second year respectively, and 1.05 per cent for scape

TABLE XII. *Variability within and between plots*

Character	Number of plants, and plot shape	No. of plots	Variability within plots				Variability between plots	
			1st year C values		2nd year C values		Greatest % difference between means	1st year 2nd year
			Range	Difference	Range	Difference		
Leaf length	408 12×34	1	24·06	—	20·76	—	—	—
	204 6×34	2	24·10-23·92	0·18	20·37-19·44	0·87	3·2	6·4
	204 12×17	2	24·41-23·95	1·36	20·36-19·72	0·64	6·3	4·8
	102 3×34	4	25·13-22·93	2·21	20·33-18·61	1·72	6·9	9·0
	102 6×17	4	27·01-20·00	7·01	22·19-17·81	4·38	11·9	11·3
	51 3×17	8	26·78-16·65	10·13	23·38-13·04	8·34	23·1	14·3
Scape length	408 12×34	1	—	—	12·51	—	—	—
	204 6×34	2	—	—	13·21-11·67	1·54	—	2·3
	204 12×17	2	—	—	12·66-12·37	0·29	—	1·4
	102 3×34	4	—	—	13·56-11·59	2·06	—	4·1
	102 6×17	4	—	—	14·18-10·81	3·37	—	3·7
	51 3×17	8	—	—	14·78-10·70	4·08	—	5·2

length. This arrangement of 102 plants may therefore be considered as a *representative sample* affording a reasonably accurate estimate of the variability of the whole population.

(b) *Variability between plots.* The mean values for the various plot arrangements were found to follow the trend of the coefficients of variation inasmuch as the differences between the extreme means increased as the number of plants in the sample decreased. These differences, expressed as percentages of their general mean values, are given for a few arrangements in Table XII. It was also observed, especially in the case of first-year plants, that, in arrangements of the same number of plants, differences were generally greater for short than for long plots, owing to the presence of a strip of lower soil fertility traversing the area in a more or less diagonal direction. Although the adoption of a lengthwise arrangement of plots reduced the effect of the fertility gradient, its influence was not entirely eliminated. For example, the scape length mean values for the four lengthwise 102-plant plots (3×34), given in order of plot position, exhibit the following trend: Plot I, $43.50 \text{ cm.} \pm 0.549$; II, 42.18 ± 0.566 ; III, 41.76 ± 0.476 ; and IV, 42.00 ± 0.494 . The significances of these values as represented by the ratios D/E_d were: comparing Plots I and II, 1.673; I and III, 2.395; I and IV, 2.029; II and III, 0.568; II and IV, 0.239; and III and IV, 0.350.

If, instead of treating the total of 408 plants as a single population, there are assumed to be four populations of 102 plants scattered as strips of seventeen plants at random in six blocks, the soil effect can be still further reduced. For example, the widest ratio D/E_d for scape length in the case of comparisons between the four lengthwise 102-plant plots was, as previously stated, 2.395, but between the four randomized populations the equivalent ratio was reduced to 1.096. It may therefore be concluded that 102 plants constitute a reliable sample of the larger population and that, although for observing the general characteristics of collections lengthwise plots (three rows of thirty-four plants) are preferable to other arrangements, greater accuracy is obtainable by adopting a randomized plot arrangement.

IV. DISCUSSION

Continuous variation is the least desirable form of diversity for use in classification. Among the larger units it may be troublesome only to a limited extent, but when small "subspecific" units are dealt with it is a problem that must constantly be faced. It is natural first to attempt to place such striking variations as may exist into

categories, but this resolves itself into a recording of the existence of either a limited number of conspicuous types, or an infinite number of phenotypes which may take their form from such unsubstantial causes as the temporary combination of characters in individual plants and the diverse modifications of local environment. The random cataloguing of phenotypes, moreover, tends to mask the real significance of character combinations, since a unit based on some character peculiarity will have the same *taxonomic* value wherever it happens to occur, whereas *biologically* its importance may depend on its proportional representation in different parts of its range. The ecotype concept postulates that the innumerable character combinations become sorted out and grouped by the environment in virtue of the constitution of the plant as a whole, and not because of any phenotypic character in particular. The concept, therefore, may elucidate a taxonomic problem which has not been fully solved by the prevailing methods. Quantitative and continuous characters assume greater importance than qualitative and discontinuous in the differentiation of races of "subspecific" rank. Moreover, cognizance has to be taken of the different combinations and proportions in which the same characters may appear locally under the selective influence of the prevailing environment. The problem, therefore, becomes one of assessing average character values and the significance of the differences between ecologically distributed populations, rather than one of describing individual variations.

In the sea plantains, discontinuous variations are few and have little importance. In this connexion the sporadic occurrence, in widely separated regions, of golden chlorophyll-deficient types may be mentioned, and also the presence, in varying numbers and colour intensities, of anthocyanin spots on the leaves of some individual plants in almost every population. Nevertheless, discontinuity in both these instances has reference only to the presence or absence of the characters. There are, however, many quantitative characters which vary continuously within populations. The ranges of these in different populations nearly always overlap, and even if they do not a series could be arranged so that there could be continuous variation throughout.

The experimental garden method may be regarded as an attempt to compare various hereditary constitutions by reducing them to a common environmental denominator. It is unnecessary to say that the environment chosen must be reasonably suited to all the material. For example, sea plantains from Greenland could not be tested at

Edinburgh because they failed to develop scapes under normal daylight, although they readily ripened seed when the period of daylight was lengthened artificially. In order to study the hereditary differences between races, it is important that errors, due to (1) ununiformity of environment and (2) methods of sampling, should either be avoided, or assessed to determine how far they may affect the results. Although absolute uniformity of environment is an unattainable ideal, yet it is possible to minimize the errors due to environmental influences by careful cultivation and planning. In the experimental garden the chief difficulties that are likely to be encountered are gradients or irregularities in soil fertility and the effects of shading or exposure. Planning might consist of some application of the yield trial technique that has been devised by statisticians for agricultural research. One difficulty, however, is that though the experimentalist may be able to cope with the individual records of one representative sample, say 100 plants, of each of the populations he wishes to compare, yet it may be impracticable for him to replicate such samples. A small uniformity trial (Table XII) shows that if the representative samples be sown side by side in single plots, as is convenient for observation, allowance must be made for a certain amount of error due to soil fertility. This error could be considerably reduced, however, if small portions of the samples were arranged at random in a series of blocks. While the comparison of duplicated samples of a population in the same season and place may show that their differences of means and variabilities are seldom significant (Table V (A and B)), similar comparisons in different localities (Table VI) or different seasons (Table VII) may indicate the magnitude of error that should be expected if dissimilarities of environment are disregarded.

Comparison of populations is completely dependent upon efficient methods of sampling the characters, but at the same time the labour of recording makes it desirable to reduce the observations upon each plant to a minimum. Quantitative characters have wide ranges of magnitude within the plant itself (Tables I and II) so that random sampling of the various parts of a plant would involve very large errors. It might be true that such errors would tend to cancel out in the estimation of population mean, but it is also requisite that a record should at least indicate the relation of an individual plant to others in respect of a character. Some form of selective sampling is therefore necessary, and in this connexion Pearson's (1901) methods of obtaining "homotypes" and of testing their validity can be

adapted to the present type of investigation (Table III). Finally it is necessary to determine the minimum number of plants that will afford a sample representative of the population, and this may be deduced from the data of a uniformity trial (Table XII).

Even when these various difficulties have been taken into account it is possible, in the plantains, to compare distinct habitat populations with some satisfaction, for differences may not only be significant, but may frequently be of magnitudes far transcending the unavoidable errors of cultivation or sampling (Table V). Thus it may be assumed that variations of a true hereditary nature are indeed present.

V. SUMMARY

1. The classification of evolutionary units on the basis of experimental facts is termed Experimental Taxonomy; it is suggested that while experimental and orthodox taxonomy are not antagonistic, any immediate attempt to absorb the experimental results into the existing system would be undesirable and would only lead to confusion.

2. The present paper is an introduction to the experimental study of *Plantago maritima* L. and closely allied species, and describes the methods adopted in studying the quantitative characters and variability of habitat populations under the comparatively uniform conditions of an experimental garden. The investigations comprise the sampling of habitats, the arrangement of habitat samples in the garden, the choice of characters, the sampling of individual plants, the examination of "errors" due to non-hereditary causes, and the formulation of methods of assessing the character values of populations.

The authors are indebted to Dr W. B. Turrill of Kew for the data which appear in Table VI, and also to the Carnegie Trust for the loan of a calculating machine.

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TERMINAL AND INITIAL PARENCHYMA
CELLS IN THE WOOD OF *TERMINALIA*
TOMENTOSA W. & A.

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(With Plates V and VI)

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I. INTRODUCTION

IN a recent note (4) it was pointed out that the so-called terminal parenchyma described by Pearson & Brown (7) and Chowdhury (3) in the wood of *Terminalia tomentosa*, W. & A. is not formed at the end of the growth season but in the beginning. This is a diffuse-porous wood and so far, occurrence of concentric parenchyma at the beginning of the growth season of a diffuse-porous wood has not been recorded, although plant anatomists are acquainted with a somewhat similar distribution in ring-porous and semi-ring-porous woods.

The object of this paper is to give a detailed study of the initial parenchyma in the wood of *Terminalia tomentosa*. It is also the intention to discuss in a general way the terms terminal and initial parenchyma as applied to the dicotyledonous woods.

II. MATERIAL AND METHOD

The description of the wood of *Terminalia tomentosa* given in this paper is based on the study of the wood specimens of this species available in the Dehra Dun collection. These specimens were collected from different parts of India, and altogether sixty-five specimens were studied and all anatomical variations within the species were noted.

In order to study the wood on living trees, two *Terminalia tomentosa* trees about 60–70 ft. in height with good crowns were selected at Lachiwala in the Dehra Dun Forest Division, 11 miles from Dehra Dun town. Small blocks containing wood, cambium and phloem were taken from these trees fortnightly or monthly during the years 1931, 1932 and 1933. Immediately after they were taken out, the blocks were fixed in absolute alcohol and brought to the laboratory, where they were later embedded in celloidin. 10–15 μ thick sections were cut for temporary as well as for permanent mounts and all the necessary data were collected from them.

III. RESULTS

The cross-section of *Terminalia tomentosa* wood shows the following anatomical structure under the microscope:

Growth rings are undulating, fairly distinct due to concentric layers of parenchyma in between late and early wood. Moreover, the demarcation of the growth ring is often made prominent by the shape and thickness of the wall of late wood fibres. The late wood fibres have thicker walls and are more tangentially flattened than those of the early wood (Pl. V, fig. 3). The number of rings per inch varies extremely. It is usually 2–15, but in a slow grown tree the number may be still higher.

Vessels are more or less uniformly distributed throughout the rings, although on rare occasions they may show a tendency to form tangential rows in the early portion of the ring (Pl. V, fig. 4). In size they show only a slight difference between the early and the late wood. This difference is more noticeable when the rings are wide than when they are narrow (Pl. V, figs. 1 and 2). Vessels are mostly single or in radial pairs of 2–3; occasionally they may be in pairs of 7–8, and are sometimes filled with blackish deposit.

Parenchyma is fairly abundant; (a) paratracheal, (b) diffuse, and (c) in concentric layers in between late and early wood. (a) Fairly conspicuous; in a row of 1–2 cells round the vessels, often extending in lateral projection on one or both sides across the rays and linking up with those extending from other vessels in the neighbourhood (Pl. V, fig. 1). Those cells which form the lateral projections are not so common when the rings are narrow (Pl. V, fig. 2). Parenchyma cells adjacent to the vessel are flattened to conform to its wall; the rest are variable in shape but often widest radially. (b) Very scanty, irregularly distributed in small patches. In size and shape they are like the paratracheal which form the lateral projections

(Pl. V, fig. 1). (c) In concentric bands of 1-2, occasionally 4 cells wide. When the early vessels happen to be near these bands, the paratracheal parenchyma cells form lateral projections on both sides of the upper end of the vessels and ultimately join with those of the concentric bands to which the lower ends of the vessels conform. Thus the parenchyma distribution round these vessels looks somewhat like a normal curve of frequency distribution (Pl. V, figs. 1, 2 and 3). In both size and shape, these cells are very similar to the paratracheal type described above. As a rule, all parenchyma cells are much larger in size than the fibres and they often contain blackish deposit.

Fibres constitute the main vertical elements of the wood. In cross-section they are somewhat circular to oval, sometimes angular, tangentially flattened especially when at the end of the growth ring (Pl. V, fig. 3); they occasionally have large lumen in the early wood but this feature is not constant. Lumen is for the most part small, often filled with blackish deposit. Gelatinous fibres are occasionally present in wide patches.

Rays are fairly numerous; cells radially elongated, occasionally compressed near the vessels, frequently filled with blackish content (Pl. V, figs. 1, 2 and 3).

In the anatomical description given above, an important point is the position which the concentric band of parenchyma holds in a growth ring, that is, whether this band is formed as the last tissue or the first tissue of a growth ring. Examination of blocks, containing wood, cambium and phloem, taken during the year 1931 show that the growth does not start till the middle of July, although the expansion of cambium cells may be noticed even a month prior to that. Study of the blocks taken out during the period of inactivity reveals that the cells formed at the end of the growth season are fibres—not parenchyma (Pl. VI, fig. 5). After the commencement of the growth, it takes some weeks for different types of cells to mature and take definite shape. By examining the mature wood of the year 1931, it has been found that the cells formed at the beginning of the growth season are those which ultimately constitute the tangential bands of parenchyma (Pl. VI, figs. 6 and 7). These observations were verified in two subsequent years, namely, 1932 and 1933. Although there was a slight difference at the time of the commencement of growth in different years, yet in all three years the first tissues to be formed during the growth season were the tangential bands of parenchyma, and the last tissues the fibres (Pl. VI, figs. 5 and 8).

It may be pointed out here that the paratracheal parenchyma of late vessels occasionally forms lateral projections of fairly extensive width. These cells due to their position are likely to be confused with those which form the tangential band at the beginning of the growth season. But careful examination will reveal that the cells forming lateral projections are not continuous—they are often intercepted by patches of thick-walled fibres—and that the vessels they enclose are slightly, if not much, smaller than those in the early wood (Pl. V, fig. 3). If attention is paid to these two points, there is little likelihood of getting the tangential bands mixed up with paratracheal projections of the late vessels. It is true that both the types may be found to coalesce but that occurs only on very rare occasions.

IV. DISCUSSION

The term terminal parenchyma was applied in the first instance to the woods of temperate regions, where by studying the wood of certain living trees it was found that parenchyma cells occur systematically at the end of each growth season. Later on, while working on the wood of tropical regions, plant anatomists have freely used this term in the case of those woods in which the tangential bands of parenchyma appeared to be distributed somewhat in the same manner as the terminal in the woods of temperate climate. In fact, in a diffuse-porous wood whenever the parenchyma cells have been found to delimit the growth ring, it has been the practice to call them terminal. In the past there could not have been any objection to this, for occurrence of parenchyma as the first tissue of a diffuse porous wood was not known. This makes it clear how Pearson & Brown⁽⁷⁾ and Chowdhury⁽⁸⁾ came to describe terminal parenchyma in the diffuse-porous wood of *Terminalia tomentosa*.

From a survey of the literature on wood anatomy, it appears that occurrence of parenchyma at the beginning of the growth season of a diffuse porous wood is recorded for the first time, and that an appropriate terminology for this type of distribution is necessary. But before taking any action in this direction, it would be desirable to examine carefully and find out whether or not any of the terms now in use for a somewhat similar structure can be applied to *Terminalia tomentosa*.

It is of interest to mention here that in response to the author's note⁽⁴⁾ on the position of concentric parenchyma in the wood of *Terminalia tomentosa* Dr Jane⁽⁵⁾ wrote to say that, according to his observations in *Cedrela odorata*, Linn., "the larger vessels of early

wood are partly embedded in parenchyma, some of which, judged by its position, was laid down rather earlier than these vessels". He also pointed out that "the vessels in the pore ring in teak (*Tectona grandis*) may be associated with parenchyma and it would be justifiable to refer to this as initial parenchyma". But both these woods are ring-porous to semi-ring-porous and as has been stated in the introduction, plant anatomists long ago noticed in them the type of parenchyma distribution pointed out by Jane. Jane's observations on the occurrence of initial parenchyma in diffuse-porous woods do not lead to any "conclusive results". In *Swietenia mahagoni* Linn. he thinks the parenchyma cells are terminal, while in *Khaya grandis* Stapf. and *Carapa guianensis* Aubl. he is not certain as to their exact position in the ring.

Now taking into consideration the ring-porous woods, the larger pores of early wood are more or less embedded in a mass of parenchyma and tracheids. By examination of these woods in the laboratory and studying them on living trees, it has been found that the inner layers of these parenchyma cells are laid down earlier than the vessels which they enclose. Some anatomists have described them as paratracheal in a general way, without attaching any importance to the position they hold in a growth ring. For example, Brown and Panshin(2) in their description of the ring-porous woods of oak, elm, etc., say "Paratracheal parenchyma intermingled with tracheids" and "Paratracheal parenchyma associated with vascular tracheids and forming tracts of conjunctive tissue between the spring wood vessels". In this connexion, Beverslius's recommendation(1), which is advocated by Pfeiffer(8), is of some interest. Based on his study of the ring-porous woods of *Cedrela odorata* Linn. and *Robinia pseudoacacia* L. Beverslius is of opinion that the parenchyma cells in the early wood should be called "marginal parenchyma". But the suitability of this term to ring-porous woods is rather doubtful, for it is altogether vague and does not indicate the position which these cells hold in a growth ring. Thus it will be seen that the terms paratracheal and marginal are too ambiguous to be applied to the parenchyma cells occurring in the early part of the ring-porous wood. As far as the diffuse-porous woods are concerned, the arguments against the application of marginal parenchyma are the same, while the term paratracheal cannot be used with any degree of accuracy, for often these parenchyma cells under consideration do not coalesce with those round the early pores and form a homogeneous mass. On the contrary, they are, for the most part, isolated from the true para-

tracheal and give the impression of a solitary metatracheal band (Pl. V, fig. 2). Now, considering the fact that these parenchyma layers are formed systematically at the beginning of each growth season in the ring-porous woods as well as in some diffuse-porous woods like *Terminalia tomentosa*, it appears justifiable to call them initial parenchyma. This term seems to be most suitable, for it indicates the time of their formation and the exact position they hold in a growth ring. Thus it will be seen that on the question of terminology the author is in entire agreement with Jane⁽⁵⁾.

Now the pertinent question arises as to the extent to which the term initial parenchyma will be applicable to other diffuse-porous woods. A definite rule for future guidance cannot be laid down at the present stage of this investigation. For some time to come it will be safer to refer to them as *initial or terminal*, and the final classification will depend on the result of the examination of woods on living trees. It may, however, be of interest to record here certain observations that have been made in this laboratory. Examination of a large number of diffuse-porous woods shows that some of them like *Michelia* and *Magnolia* undoubtedly have terminal parenchyma, while there are others which exhibit parenchyma distribution somewhat like the initial type of *Terminalia tomentosa*. The difference between the two types appears to be mainly confined to their shape and size. As a rule, the terminal type is tangentially flattened and rectangular in shape, while the initial shows variation in shape, from rectangular to triangular but for the most part widest stradially. Moreover, the fibres in the neighbourhood of these parenchyma cells indicate the position of the latter in the growth ring.

In the course of this investigation, certain interesting results have been obtained which indicate the probable line of evolution from ring-porous to diffuse-porous woods or vice versa. Further investigation in this line is being continued, and it is hoped to discuss the whole question fully in a succeeding paper.

Although much has been written upon the different types of vertical parenchyma in dicotyledonous woods, yet irregularities in their application are often met with in the literature on plant anatomy. To cite a few examples, Kanehira⁽⁶⁾ is the only worker to describe terminal parenchyma in some oaks, while Pearson & Brown⁽⁷⁾ and Brown & Panshin⁽²⁾ mention terminal parenchyma in *Juglans regia* L., but not in *Juglans nigra* L. although these species show very little difference in parenchyma distribution. All these irregularities appear to be due to the confusion over the

characteristics on which classification is based. Parenchyma cells have been classified into paratracheal, metatracheal and diffuse based on the definite arrangement and pattern which they form in the wood. They are also referred to as terminal and initial according to the position they hold in a growth ring. Every endeavour should be made not to confuse one type with the other. For instance, when parenchyma cells are found to be uniformly paratracheal throughout the growth ring, from the early to the late wood, those of the extreme early or extreme late wood should not be called initial or terminal instead of paratracheal. After all, the same pattern is found throughout the growth ring and there is no justification for calling them by separate names by virtue of their mere position. Uniformity in description can only be maintained if those at the extreme early and extreme late wood are called paratracheal. The same rule is applicable to the terms metatracheal and diffuse. In view of the above consideration, application of the term terminal will be restricted to the parenchyma cells arranged at the end of the growth ring in a pattern which is not repeated in any other portion of the ring. The same restriction may also be applied to the initial parenchyma, the pattern and arrangement of which are not found in any other part of the growth ring except at the beginning.

V. SUMMARY

1. A study of the wood of *Terminalia tomentosa* W. & A. on living trees shows that the concentric bands of parenchyma which delimit the growth rings are formed as the first tissue of a seasonal growth. It is, therefore, incorrect to call them terminal parenchyma.
2. Occurrence of parenchyma cells at the extreme early wood of a diffuse-porous wood is recorded for the first time, and it is proposed to call them initial parenchyma, indicating their position in a growth ring.
3. A somewhat similar distribution has been known for a long time to occur in ring-porous woods but so far no special nomenclature has been applied to it. For the sake of uniformity in description it is also proposed to call it initial parenchyma.
4. In diffuse-porous woods when the exact position of concentric parenchyma cells, which delimit the growth rings, is difficult to determine, it is advisable to describe them as *initial or terminal*. The final classification will always depend on the study of these woods on living trees.

5. In view of the discrepancies in the use of the terms paratracheal, metatracheal, diffuse and terminal, the characteristics on which the classification of the parenchyma cells are based are discussed in detail and certain suggestions are made regarding the restricted use of these terms in order to standardize the anatomical description of woods.

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EXPLANATION OF PLATES V AND VI

PLATE V

Terminalia tomentosa W. & A.

Fig. 1. Transverse section of a wide growth ring, showing variation in the size of the vessels and distribution of the parenchyma cells. $\times 20$.

Fig. 2. Transverse section of narrow growth rings, showing little difference in the size of the vessels and scanty development of parenchyma cells. $\times 20$.

Fig. 3. Transverse section of the junction of two growth rings, showing initial parenchyma in the early wood and paratracheal projections from the late vessels. $\times 80$.

Fig. 4. Transverse section showing the grouping of vessels in the extreme early wood somewhat like a ring-porous wood. $\times 30$.

PLATE VI

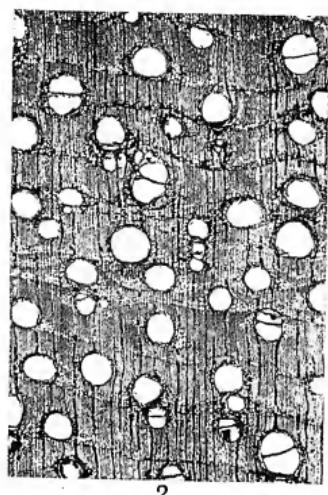
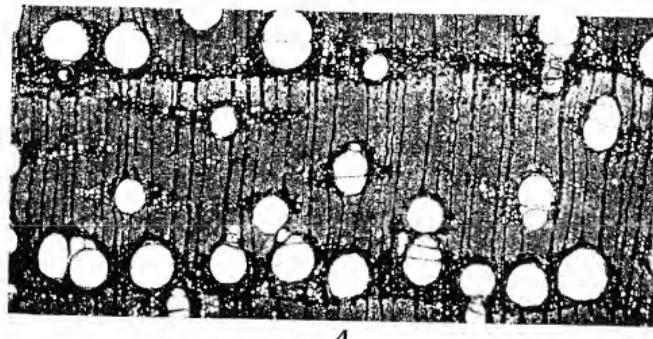
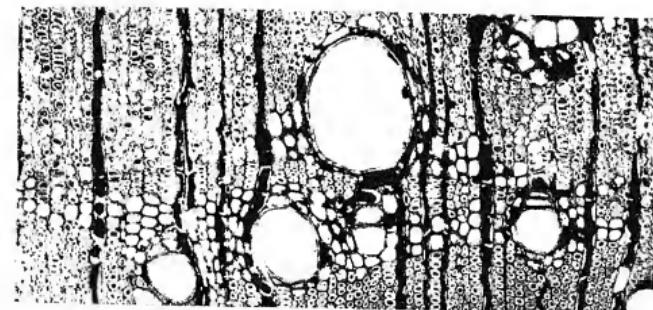
Terminalia tomentosa W. & A.

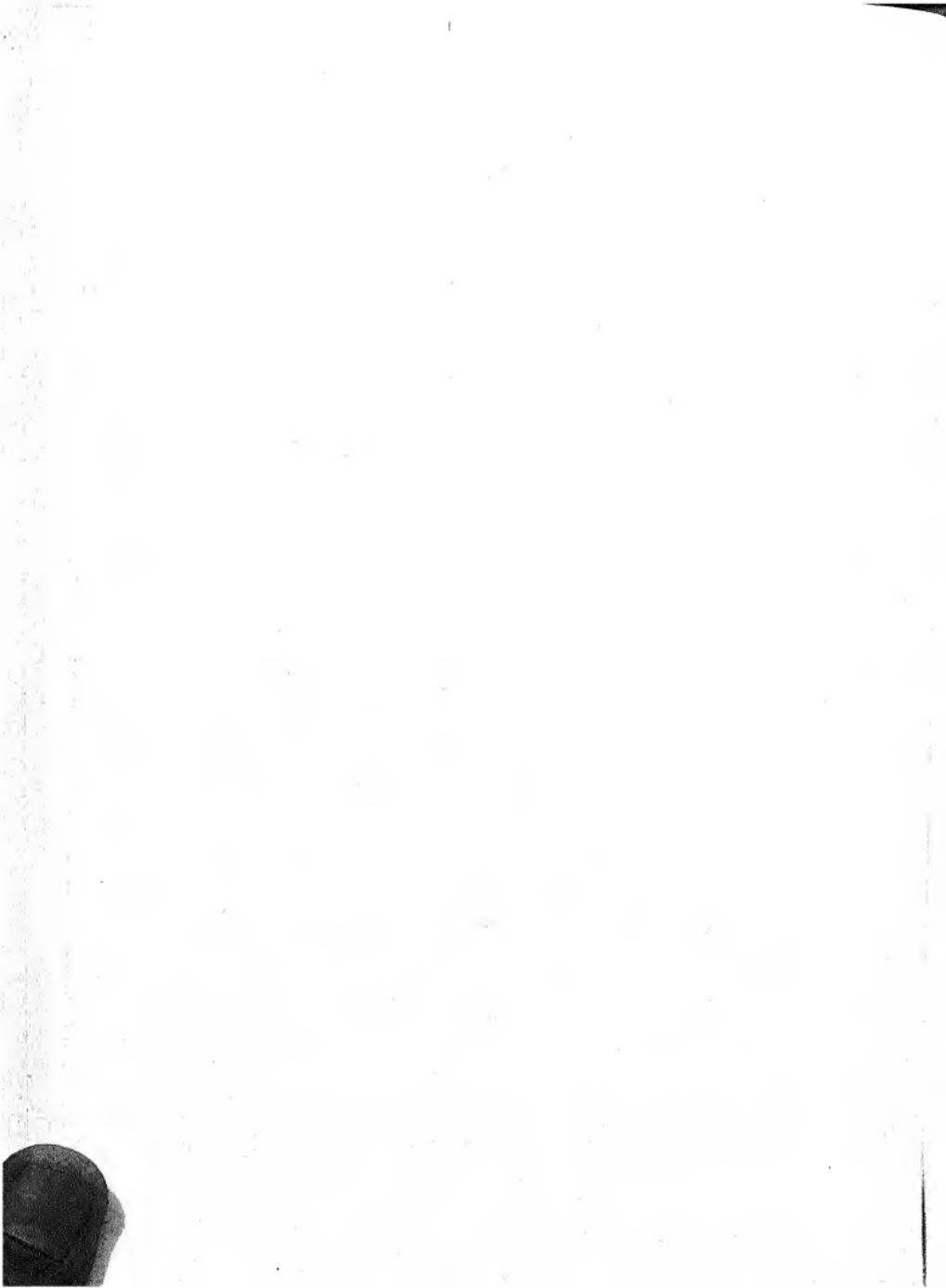
Fig. 5. Tree 2, 17 June 1933, showing dormant cambium and absence of concentric band of parenchyma. $\times 60$.

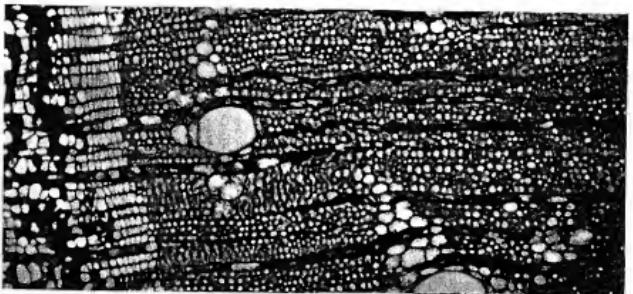
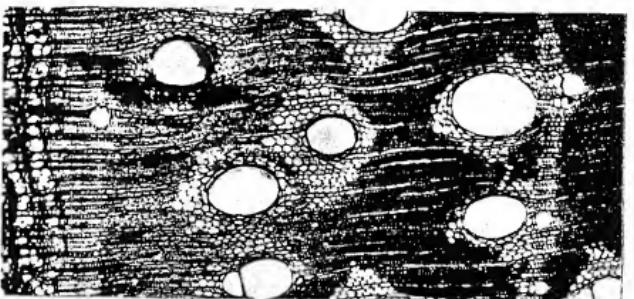
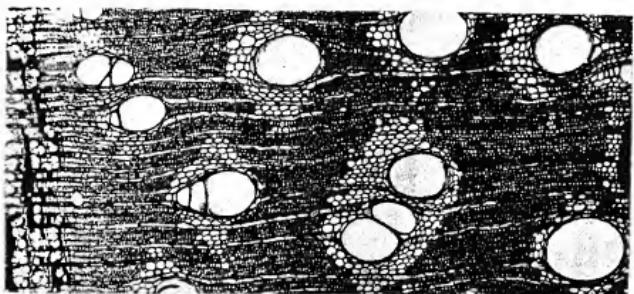
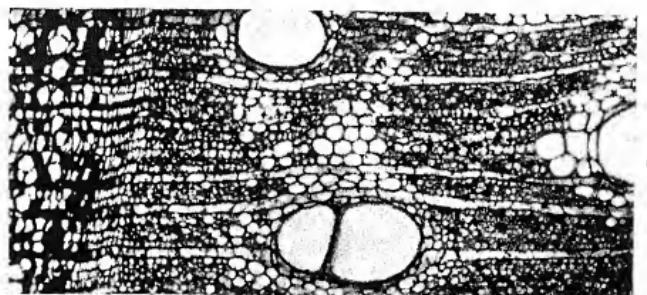
Fig. 6. Tree 1, 30 August 1932, showing the active cambium and a concentric band of parenchyma formed at the beginning of the season. $\times 30$.

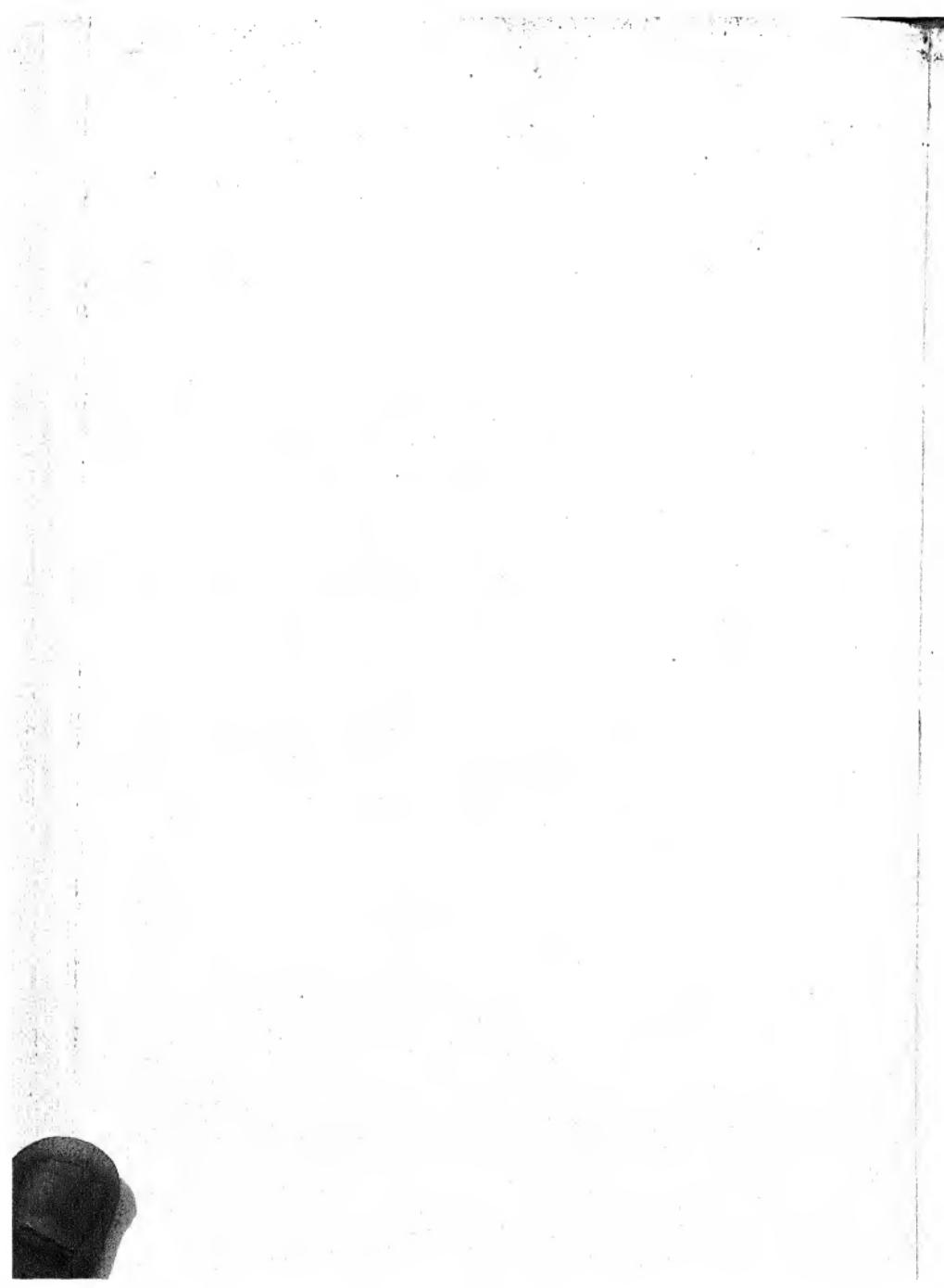
Fig. 7. Tree 1, 15 October 1932, showing concentric band of parenchyma, active cambium and fairly mature cells of the wood near the cambium. $\times 30$.

Fig. 8. Tree 1, 15 November 1932, showing dormant cambium and no parenchyma band formed at the end of the growth season. $\times 60$.









STUDIES IN THE AUTECOLOGY OF
CLADIUM MARISCUS R.BR.

II. ENVIRONMENTAL CONDITIONS AT WICKEN FEN,
 WITH SPECIAL REFERENCE TO SOIL TEMPERA-
 TURES AND THE SOIL ATMOSPHERE

By VERONA M. CONWAY

Yarrow Research Student of Girton College

(With 5 figures (1 folding) in the text)

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INTRODUCTION

CLADIUM MARISCUS has been studied as it grows at Wicken Fen, Cambs, and while some account has already been given of the structure and development of the species itself (Conway, 1936), the present paper deals with certain characteristics of the environment, more especially the edaphic conditions. The behaviour of the water table in the fen is already known in some detail, but until now no information has been available as to soil temperatures and the composition of the soil atmosphere. Some general information on these subjects is clearly desirable in studying the autecology of a fen species, but the data are discussed here at rather more length than is strictly necessary for this purpose, because they seem of interest for their own sake, in showing the characteristic behaviour of the fen peat.

I. GENERAL CONDITIONS

The fen lies about 10 miles north-north-east of Cambridge and thus it belongs to the region with an annual rainfall of about 24 in. The general climate of East Anglia has been described by Salisbury (1931) and Watt (1936), and there is no reason to suppose that climatic conditions at Wicken are exceptional. The data on climatic conditions during the period 1934-6 are confined to certain measurements of air temperatures which will be referred to later, and daily rainfall measurements taken at Fordham, $\frac{1}{2}$ miles away from Wicken, and kindly provided by Mr W. V. Bloom. It is the edaphic conditions that are of especial interest, because it is these that are in effect maintained by the human management of the fen. While the surrounding land is drained and cultivated, the fen is kept artificially undrained in order to maintain the plant species which would occur wild in a wet habitat of this sort. The soil is black fen peat, which generally gives a water content of about 80 per cent of the volume in a fresh state and an air-space volume up to 11 per cent. The dried soil consists of 60-80 per cent by weight of organic material.¹ The ground water of the fen is derived from calcareous strata and is always alkaline. The alkalinity causes the very complete breakdown of the plant debris from which the peat is formed, and the fertility of the East Anglian fen peats and the basiphilous flora which they bear are well known and have been frequently described and referred to by previous workers. This is one of the two main edaphic factors influencing the fen vegetation. The other is the high level of the water table relative to the soil surface with, as a result, a very high moisture content of the soil in which the plants are growing.

The changes in the water-table level have been investigated by Godwin (1931) and Godwin & Bharucha (1932), and they have shown that in a normal winter the water-level is close to or above the soil surface all over the fen, while in the summer it is near the surface at the margins of the fen (say 1 ft. or less), while it is lower in the centre of the fen, falling to a depth of 4 ft. or more. Even at the driest seasons, however, no part of the fen ever has soil which is dry to the touch. Whether or not any of the fen plants suffer from the lowering of the water table in the summer is a point of some interest, which will become more prominent when the water relationships of *Cladonia Mariscus* are discussed. The changes in the water-level during the

¹ This fact is derived from the M.Sc. thesis written by F. R. Bharucha and now in the possession of the University of Cambridge.

period of investigation have been followed by means of the permanent water-level recorders set up by Godwin in 1928 and described by him in 1931. One of these is on the margin of the fen, close to a dyke; the other in the centre. The records give an approximate idea of the rainfall, and there is a close agreement with the more accurate data obtained from Fordham, except for the smallest showers.

The high moisture content of the soil has a marked effect on certain of its characteristics, when it is compared with the soil of average mesophytic conditions elsewhere in Britain. If only qualitative estimates were concerned, such a remark would be almost too obvious to be made, but the results about to be described do give a quantitative measure of the differences in two of the soil characters, namely soil atmosphere and soil temperature.

II. SOIL TEMPERATURES

Since June 1934 permanent records of soil temperatures have been obtained from two thermographs set up in the middle of the fen in the locality A from which samples of soil atmosphere were taken occasionally. They were also close to one of the permanent water-level recorders. The instruments were of the standard design made by the Cambridge Instrument Co. The thermometer consists of a metal bulb about 15 cm. long and 2 cm. in diameter, full of mercury. The mercury passes into a very fine metal capillary 6 ft. in length, and the pressure variations due to expansion or contraction of the mercury are transmitted to the pen arm. Each instrument contains two such thermometer bulbs, each working a separate pen. Four bulbs were thus available, and they were arranged vertically above one another. The uppermost was supported about 5 cm. above the soil surface and parallel with it. The next was placed at the surface of the soil, embedded in the loose debris of dead leaves, etc., with the lower surface of the bulb pressed closely against the soil. The next was buried at a depth of 15 cm. and the last at 30 cm. below the surface. The pit which had to be dug to insert the two latter was filled up and trodden down firmly and the plants which had been dug up were carefully replaced. The vegetation at this point is of the "Mixed Sedge" type with bushes just starting to colonize. The bushes are not yet over 5 ft. in height. There is thus quite a dense vegetation and the uppermost thermometer never registers sun temperatures. Owing to the 6 ft. of capillary by which the bulbs are attached to the main instruments, the latter can be set up far enough away to prevent

any disturbance of the soil or vegetation near the bulbs so that the latter may be considered to give a picture of natural fen conditions.

The type of record obtained is illustrated by the accompanying figures (Figs. 1 and 2). The first shows the graphs for 6-9 July 1934. The original records, of course, consisted of two sheets, but all four curves have been traced on to one sheet here for convenience. The days represented here cover one of the finest weather periods that has occurred since the recorders were set up. The second figure (Fig. 2) illustrates the opposite extreme of winter weather, for 7-14 January, 1935.

An attempt has been made in the accompanying graph (Fig. 3) to summarize some of the main data given by the record sheets for the year 1935. The upper set of vertical lines refers to the air thermometer, the next to the surface thermometer and the two lower lines to those at 15 and 30 cm. respectively. The temperature scale is the same for all though each has a different zero for the sake of clarity. Each vertical line in the air thermometer graph joins the maximum for any particular day with the minimum for the night following, so that the graph gives no information as to the hour at which these values occurred. In cases where there was no maximum or minimum value, that is, when the daily periodicity was overridden by a continuous upward or downward drift, an oblique line has been drawn in the appropriate direction. The same method is used for the surface thermometer. The two buried thermometers showed a daily fluctuation of such small range that it was possible to transcribe the continuous curves from the original records. The accuracy of reproduction is, of course, limited by the closeness of the time scale. The interruptions in these curves were due to technical hitches in the working of one of the recorders. During the gap from 14 to 22 July, the limits of travel of the pens could be ascertained, and these have been indicated by horizontal lines.

It is obvious from the graph to what a large extent the daily fluctuations in air temperature are damped down at the soil surface and at 30 cm. depth they are imperceptible except in the very hottest weather such as is illustrated in Fig. 1. Not only is the daily range much smaller but so also is the annual range, which for the air thermometer is from -7 to 25° C., while at 30 cm. it is from 4 to 14° C., and none of the thermometers shows temperature below zero except the air thermometer. Indeed, the buried ones never approach within 3° of zero. The two lower curves show a very close agreement, and it is rare to find a difference of more than 1° between

them. The gradient between them is reversed in spring and autumn, at which periods the two curves are practically superposed. What one might call the seasonal cross-over dates from 1935 are about 17 March and 4 October, though minor fluctuations may cause temporary reversals of the gradient at other times.

The problems of soil temperatures have been discussed, and recent work summarized, in a chapter of Keen's *Physical Properties of the Soil*. The matter is approached by considering the propagation of waves of temperature increase or decrease through the medium of the soil. Besides the irregular impulses due to fluctuating weather conditions, there are two obviously periodic changes, the daily and the annual. Obviously the deeper any point in the soil is the later will the maximum or minimum of the wave reach it. This lag is apparent in the Wicken data for both the daily and the annual variation. The former is illustrated by the figure for 5-12 July, 1934, and the latter by the graph for 1935. In the annual variation it is not so much the actual dates of reaching the peak values which lag, but the date which represents the centre of symmetry of the smoothed curves which could be obtained from the actual records. A progressively diminishing amplitude would be expected in a wave propagated through a material medium and this effect is strongly shown by the Wicken data, as previously mentioned.

Keen has shown how the characteristics of a soil in relation to temperature may be expressed quantitatively and he obtains the equation

$$\frac{K}{c} \frac{d^2\theta}{dx^2} = \frac{d\theta}{dt},$$

for the change of temperature (θ) at a distance x from the source of heat supply. K is the heat conductivity and c the specific heat of the medium. The quantity K/c or k is called the diffusivity of the soil and is a convenient method of expressing the ease with which a soil will transmit temperature changes. The value of k varies for any one soil according to its condition and especially its degree of moisture. Keen gives figures to show that there is an optimum moisture content (about 10 per cent). Below this the porous nature of the soil gives K , and hence k , a low value, while a greater amount of moisture increases c more than it increases K , so that the value of k falls. Besides the effect of moisture on the true value of k , water movements in the soil may cause an apparent change on one hand by lowering the temperature by evaporation, and on the other by the mass movement of water and the heat it carries, in percolation through

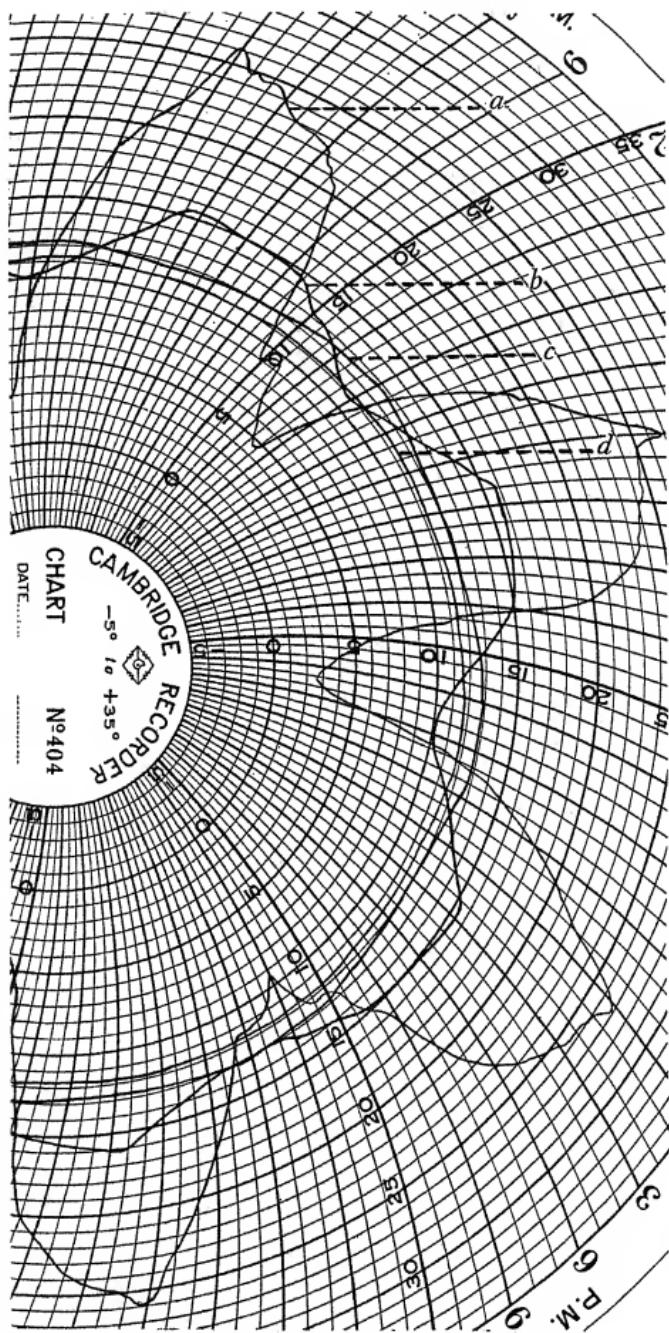


Fig. I.

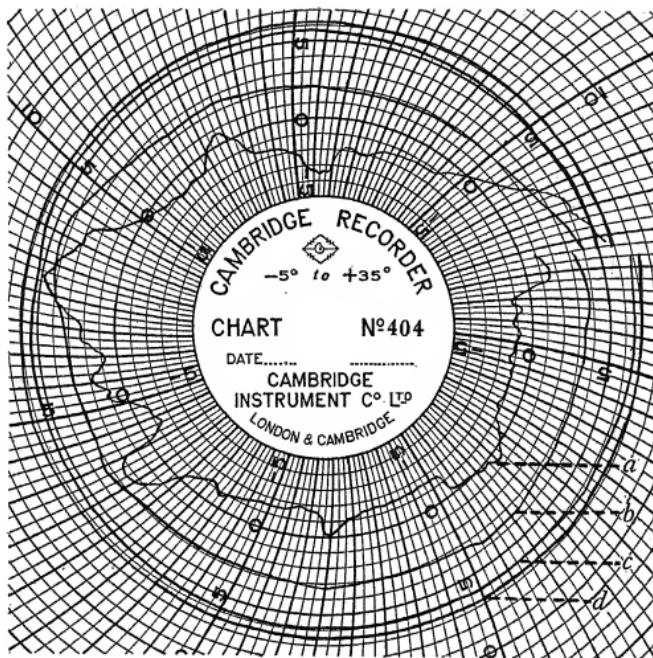


Fig. 2.

Fig. 1. Tracings of temperature records for 6 to 9 July 1934.

- a. Thermometer bulb 5 cm. above soil surface.
- b. Thermometer bulb lying in soil surface.
- c. Thermometer bulb 15 cm. below soil surface.
- d. Thermometer bulb 30 cm. below soil surface.

Fig 2. Tracings for 7 to 14 Jan. 1935. Lettering as above.

the soil. Keen quotes the work of Callendar & McCleod in Montreal; they applied the equation given above to obtain values of k , by observing temperatures over a range of depths and times. They obtained values of k ranging from 0.001 to 0.01 (c.g.s. units) with one exceptional value of 0.5 after very heavy rainfall, with presumably rapid percolation. The minimum value of k occurred at a season when, owing to frost, no percolation could occur.

Continuous records of soil temperatures taken at Rothamsted have been obtained by Keen & Russell (1921) and are interesting to compare with those from Wicken. The general features with regard to the lag in reaching peak values and the decrease in amplitude of fluctuation with increasing depth are the same in both cases. The amplitude at corresponding depths is, however, much greater at Rothamsted. At 6 in. (corresponding to the 15 cm. thermometer at Wicken) there is an average daily range of 2° C. in the winter, whereas at Wicken such a range is not nearly achieved even in the summer. The summer maximum values are also higher, namely in the region of 21° C., as compared with 16° C. at Wicken. This difference may be partly explained by the fact that the Rothamsted values are for bare soil, so that the fluctuations applied to the soil surface are very much greater. It is, however, also due to a difference in the soil itself.

If the notion of a temperature wave which passes through a point at a depth x be expressed by the ordinary equation

$$\theta = \theta_0 e^{-\frac{2\pi x}{\lambda} \sin 2\pi \left(\frac{t}{T} - \frac{x}{\lambda} \right)},$$

then combining this with the previous equation gives

$$k = \frac{\lambda^2}{4\pi T};$$

T is the period of the wave motion, λ its wave-length. If θ_1 and θ_2 are the amplitudes of the wave at depths x_1 and x_2 then

$$\frac{\theta_1}{\theta_2} = e^{-2\pi(x_1-x_2)/\lambda}$$

from the previous equation for the wave motion; or, substituting for λ ,

$$\frac{\theta_1}{\theta_2} = e^{-(x_1-x_2)\sqrt{\frac{\pi}{Tk}}}.$$

Keen applies this expression to calculate k from the Rothamsted data by comparing the amplitudes of the daily wave at 4 in. depth and 8 in. depth and using the mean value of $\frac{\theta_1}{\theta_2}$ for a large number of ob-

servations. This gives a value of k for Rothamsted soils of 0.00408 c.g.s. units.

It is not possible to use this method for the daily wave in dealing with the Wicken data because the 30 cm. amplitude is too small to be read accurately. Nor would it be legitimate to compare the 15 cm. depth with the surface since the latter is not strictly in the transmitting medium. It does, however, seem possible to attempt at any rate a rough estimate for k by using the equation

$$k = \frac{\lambda^2}{4\pi T}.$$

If there is a lag of ϕ hours at a depth x in attaining the maximum of any one oscillation, then the wave-length is given by

$$\lambda = \frac{Tx}{P}.$$

On many days during the summer months it is possible to read off from the records of the 15 cm. thermometer the time of reaching the daily maximum to within an hour, while the time of the corresponding maximum at the surface can be read to the nearest half-hour as a rule. The accompanying table gives the times of attaining the maximum temperature in any one daily wave for all the dates during 1935 on which the maxima were sufficiently well marked, and the corresponding values of k are worked out and plotted in the graph (Fig. 4). Only those dates have been used for which the surface temperature showed an increase to a maximum after a definite minimum the night before, so that the equation for a sine curve should not be too remote from actuality. Even so, the shape of the "wave" varies considerably from day to day, and this probably accounts to a considerable extent for the scatter of the points in the graph over and above the inaccuracies introduced by uncertainty of the exact times of the maxima on the curves.

The four points which seem to lie outside the general range of distribution on 7, 8, and 31 July and 1 August are associated with curves at the surface which reach their maximum later in the day than is usual, and are thus asymmetrical. The deviations from symmetry in these cases, however, do not make the curves appear outstandingly irregular in shape compared with those for other days so that it does not seem justifiable to leave them out of the table on that account. It is quite possible that some condition obtained on those dates which genuinely caused unusually high diffusivity. Since the value of 1.86 would have been reduced to 1.47 if the surface

TABLE I

Date	Time of temp.		k in c.g.s. units $\times 10^3$	$k = \frac{3}{8\pi d^2}$	Date	Time of temp.		Difference in hours
	Surface	15 cm. depth				max. in hours after noon	Surface	
11 April	2	21	19	0·33	24 June	5·5	18·5	13
12 "	6·5	18·5	12	0·83	"	4	20	16
14 "	2	16·5	14·5	0·57	"	3	17	14
17 "	6	22	16	0·47	"	27	4·5	13·5
19 "	4	20	16	0·47	"	28	5·5	15·5
20 "	2	17	15	0·53	"	30	5·5	17·5
21 "	3·5	18·5	15	0·53	2 July	6	17·5	11
22 "	5	17·5	12·5	0·76	"	6·5	17	10·5
29 "	5	21	16	0·47	"	3 "	18·5	13
9 May	3	16·5	13·5	0·66	"	5·5	18	0·50
10 "	3	19·5	16·5	0·44	"	5 "	15·5	10
20 "	2	20	18	0·37	"	7·5	15·5	8
21 "	3·5	19·5	16	0·47	"	7	15	8
24 "	3	18·5	15·5	0·50	"	8	18·5	10·5
25 "	2·5	18·5	16	0·47	"	7	17·5	10·5
30 "	0·5	17·0	16·5	0·44	"	5	18	13
31 "	3	18·5	15·5	0·50	31 "	7·5	15·5	8
2 June	6·5	19·5	13	0·71	1 Aug.	7·5	15·5	8
3 "	1·5	17	15·5	0·50	"	7·5	16·5	9
5 "	6	19	13	0·71	"	8	18	10
7 "	2	19	17	0·41	"	4	17·5	10
8 "	3	18·5	15·5	0·50	"	5	17·5	11
11 "	3·5	18	15·5	0·50	"	6	18	0·99
12 "	3	17·5	14·5	0·57	"	8	19·5	0·90
14 "	1·5	19	17·5	0·39	"	10	15·5	13·3
18 "	5·5	20	14·5	0·57	"	18	6·5	0·99
20 "	6·5	20·5	14	0·61	"	20	6	11·9
21 "	6	17·5	11·5	0·90	"	21	6	0·83
22 "	3	19	16	0·47	"	22	5·5	0·71
23 "	1·5	19·5	18	0·37	"	25	6	0·99

$$k \text{ in c.g.s.} \\ \text{units } \times 10^3 \\ h = \frac{3}{8\pi d^2}$$

$$h = \frac{3}{8\pi d^2}$$

maximum had been taken as occurring an hour earlier, that is, more nearly at the midpoint of the curve, the writer inclines to the view that the explanation does lie in the shape of the curve rather than in some peculiar physical condition. Yet, however one interprets these extremes, there is no doubt that on the whole the points are scattered round an average value, which rises from about 0.0005 in the spring to about 0.0012 in the late summer. It may be mentioned that a value of k worked out from the lag in the annual tempera-

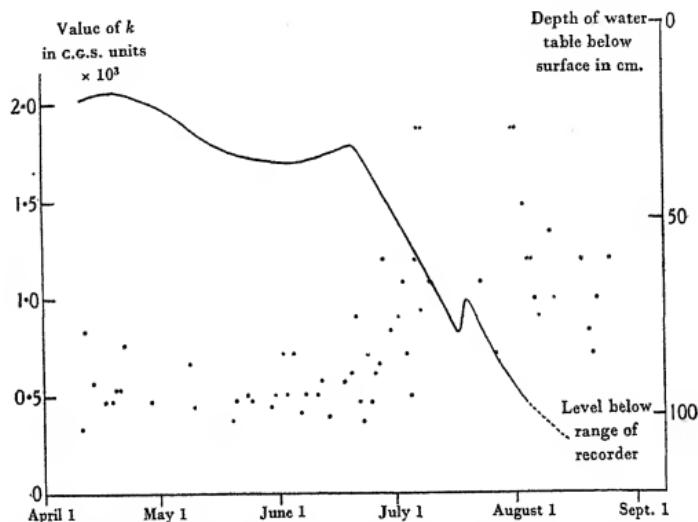


Fig. 4. Graph showing variation in temperature diffusivity (k) of the soil at Wicken Fen, and the level of the water table during the summer of 1935.

Isolated points = value of k .

Continuous line = depth of water table.

ture wave is 0.002, which, considering the roughness of the estimate, is quite an interesting confirmation.

It appears therefore that k for Wicken soil in the summer has values between 0.0003 and 0.002, with an average value of about 0.0007; that is, the range lies below the values obtained either at Rothamsted or Montreal. Keen's graphs for the variation of k with soil moisture do not go above 20 per cent moisture, but at this point the curves are sloping downwards rapidly so that if extrapolated they would cut the zero line at 30–35 per cent moisture. Hence the very low values for Wicken may well be associated with a degree of

soil moisture much above the average. The same fact might explain the general rise in values of k during the later part of the summer which is shown, since at this time the water table falls to its maximum depth below the surface, and the increased air temperature and greater water loss from the vegetation must have a considerable effect in reducing the soil moisture content and so increasing k .

A cause which may contribute to lower values of k is the different chemical composition of peat itself compared with mineral soil particles; the heat conductivity (K) for the latter may be appreciably higher than for the organic compounds which are derived from plant material. The cause of a low value of k is not, however, of primary interest to the ecologist: to him it is the fact that the value is low that expresses one of the conditions which make up the environment of the plants growing in this soil. In other words, temperature conditions in the peat soil are more uniform than in mineral soils, and one consequence of this is that the drying out of the soil in summer by high temperatures in the surface layers is made less likely, even though the water table falls quite low. The value of k is, of course, only derived from the summer temperature records, but the temperature curves for the whole year do not suggest any change in the behaviour in the winter, so that the chances of frost occurring in the soil are negligible. If Rothamsted soils are considered as bearing a fairly close resemblance to the majority of natural British soils, at any rate in chemical composition, it would appear that the Wicken soil offers temperature conditions which would be more favourable to species whose underground parts suffered from frost than those afforded in many parts of Britain. Geophytes and hemi-cryptophytes would be especially favoured.

There must be many other deductions which could be made from the extensive primary data which are provided by continuous records. They would be concerned to a large extent with the details of the causal relationships between the air temperature and the soil temperature and would therefore be mainly of interest to the physicist. The causes determining the air temperature would be of interest and have been discussed by Keen & Russell in relation to the Rothamsted data, but as no meteorological data is available at Wicken this side of the question cannot be touched, apart from mentioning the obvious effect of cloud in lessening the difference between day and night temperature.

III. COMPOSITION OF THE SOIL ATMOSPHERE

(a) *Methods*

Samples of the soil atmosphere were obtained at intervals from certain points in the fen and brought back to the laboratory for analysis of carbon dioxide and oxygen. In order to do this, a pit of about 80 cm. depth was dug out at the required place, and at specified depths in the sides of the pit horizontal tunnels were bored out of about an inch in diameter and 6 in. long. Into these were inserted cylinders of perforated copper closed at both ends with corks. Through one of the corks passed a piece of glass tubing bent at right angles close to the cork, so that when the cylinder was inserted into the hole in the soil the glass tube passed up vertically beside the side wall of the pit. Three such cylinders were inserted one on each of three sides of the pit; their depths were 20, 40 and 60 cm. respectively. The ends of the glass tubing projecting above the soil surface were closed by rubber tubing and screw clips. The soil was then returned to the pit, and was pressed and stamped in as firmly as possible so that it should not be more porous than the undisturbed soil around it. The space inside the copper cylinders comes to contain a gas which is in equilibrium with that occupying the pore space of the soil, provided there is no leakage through the glass tube. When once this has been set up samples of the gas can be withdrawn at intervals when required by suction through the tube.

It may be objected that to dig out such a pit must make conditions abnormal, so that the gas analysed does not represent the true soil atmosphere. It is probable, however, that if this were the case the disturbed soil would gradually return to normal conditions over the course of years, so that there would be a gradual drift away from the composition of atmospheric air towards that characteristic of the soil atmosphere, i.e. the carbon dioxide values should show a steady upward drift superposed on any seasonal changes. Such a drift was present in trial pits made by Bharucha in which there was insufficient consolidation (Bharucha, M.Sc. thesis). Nothing of the kind has been observed in the present work and hence it has been assumed that the method is valid.

The gas samples were drawn out into special gas sample tubes of about 80 c.c. capacity by applying a reduced pressure with a mercury reservoir arrangement. Precautions were taken to prevent contamination by atmospheric air during the process. The gas sampling tube was taken back to the laboratory and the gas analysed with a small

(portable) Haldane apparatus. Two analyses were made of each sample for carbon dioxide and oxygen, except in a few cases where, owing to lack of time or faults in the Haldane apparatus, only carbon dioxide was determined.

Results were obtained from two places on the fen referred to as *A* and *B*. *B* was at the margin, about 20 ft. away from the bank of a dyke, and except in the hottest season gas could only be obtained from the 20 cm. depth, the 40 and 60 cm. cylinders lying below the water table. It was from this point (*B* 20) that an attempt was made to obtain a picture of the seasonal variation in carbon dioxide values. The aim was to take readings once a fortnight, but this could not always be carried out, especially in the summer of 1935. The samples were always obtained at about the same time of day, between 10 a.m. and 12 noon, so that if there is any daily rhythm this has been avoided. The results for carbon dioxide values are shown on the accompanying graph (Fig. 5) as a continuous line. During the summer of 1934 several samples were taken from *B* 40 and are shown on the graph as encircled spots. Readings were taken much more sporadically from the second locality, *A*, which was situated in the middle of the fen. Most of them were for 20 cm. depth, and these are also shown on the graph.

The graph shows a number of other quantities in addition. The points showing the deviation of the sum of carbon dioxide and oxygen from 21 per cent refer to *B* 20. The soil temperature graph is in two parts. The first, shown as isolated crosses, are the readings from a mercury thermometer placed in a horizontal metal tube which runs into the soil at a depth of 20 cm. from the side of a pit about a foot square and 2 ft. deep, covered by a wooden lid. These cannot be taken to give more than a rough idea of the drift in soil temperature. The second part is a curve taken from the records of the permanent temperature recorder for a depth of 15 cm. Both the pit and the recorders are situated at the locality *A*, and hence there must be some caution in applying these results in interpreting the curve of carbon dioxide at *B* 20, since *B* is some 300 yards away from *A* and differs in being close to the dyke and having therefore on the average a higher water table.

There is no reason to suppose, however, that the direction of temperature change in the two places would differ except over a range of a few hours, though the absolute magnitudes might differ somewhat in the two places.

The rainfall curves consist of a series of points corresponding to

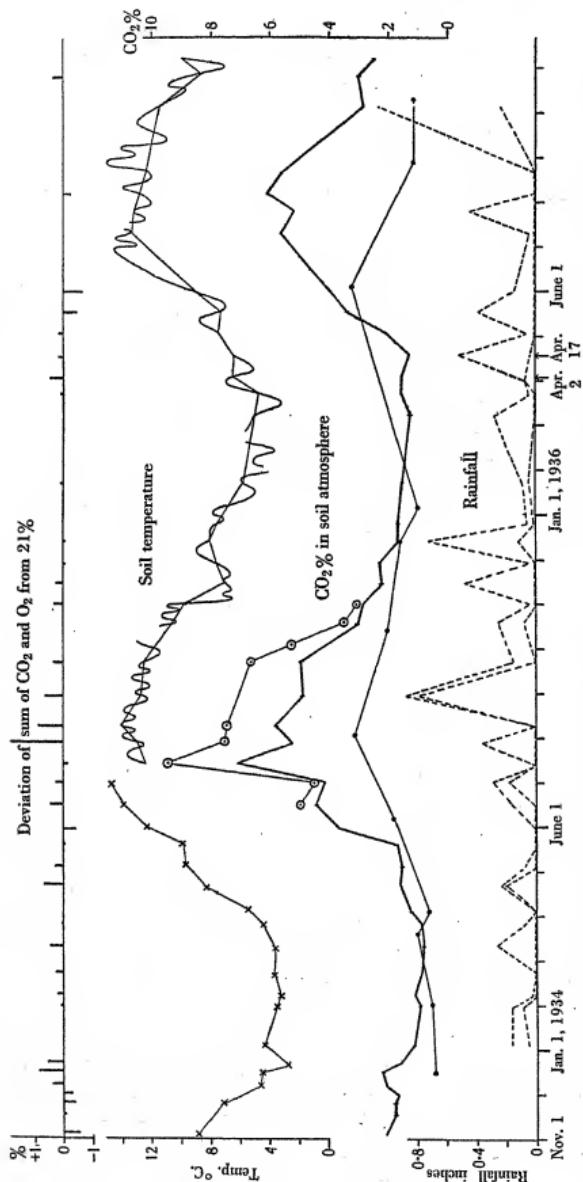


Fig. 5. Results of investigations of the soil atmosphere. — Carbon dioxide percentage at 20 cm. depth at locality B (B 20). \circ — \odot Carbon dioxide percentage at 40 cm. depth at locality B. \rightarrow Carbon dioxide percentage at 20 cm. depth at locality A. \times — \times Soil temperature at 20 cm. depth in thermometer pit, on day of taking B 20 sample. \curvearrowright Soil temperature at 15 cm. depth, given by continuous recorder. — Mean temperature at 15 cm. depth for 24 hours preceding time of taking B 20 sample (derived from continuous recorder). ----- Rainfall curves. Upper gives total rainfall for the week, lower for the 24 hours preceding time of taking B 20 sample.

the points on the *B* 20 curve, the upper curve representing the rainfall for the 7 days preceding the date on which the gas sample was taken, the lower curve for the 24 hours preceding.

(b) *Results*

Perhaps the most striking character of the carbon dioxide values is the marked seasonal drift, corresponding to the general drift of soil and air temperatures. The drift is the same for *A* 20 and *B* 40 as it is for *B* 20, the differences between them lying in the absolute carbon dioxide values. The maximum values of 7.1 and 9.4 per cent for *B* 20 and *B* 40 are also very high compared with those for ordinary moist soil, as will be mentioned later. Even the maximum of 3.3 per cent for *A* 20 is relatively high. At *B* it is evident that the carbon dioxide value increases with depth and the same is true at *A*: for instance, when on 2 August 1934 *A* 20 gave 3.20 per cent carbon dioxide, *A* 40 gave 4.12 per cent. With regard to the generally higher values at *B* than at *A*, it can only be suggested that the higher values are associated with a higher summer water table, i.e. that there is a direct correlation between soil moisture and carbon dioxide production.

The values for the sum of carbon dioxide plus oxygen percentage do not diverge very markedly from 21 per cent, which suggests that anaerobic processes do not play a large part in the breakdown of soil carbon compounds, though it is not suggested that anaerobic conditions are necessarily absent from the soil. The sum tends to be more than 21 per cent though not invariably. The values do not show any striking correlations either with carbon dioxide values or with temperature and rainfall curves. Some but not all of the highest values do follow rainy weeks, but the converse does not hold.

The carbon dioxide curve for *B* 20 is not nearly so continuous as might be desired, but even so it may be possible to suggest interpretations for the minor fluctuations as well as for the general seasonal drift. These fluctuations are outside the experimental error of the analysis; the latter is shown for each point as a vertical line joining the two carbon dioxide values, of which the mean is the point on the curve.

It is apparent from the graph that a rainy week before taking the sample causes a relatively lowered carbon dioxide percentage, at any rate during the summer months and usually in the winter also. Rainfall might have a direct effect by taking up carbon dioxide into solution during its passage through the soil, or it might affect the activity of soil organisms or of soil reactions in general by altering conditions

temporarily. On the other hand, there is a strong negative correlation between rainfall and air temperature, and hence soil temperature, so that it is quite possible that the lowered values in the carbon dioxide graph are due only to lowered temperatures, and rainfall has no direct effect. Whether this is so or not is impossible to decide from the data because the carbon dioxide curve is too coarse for the purpose. It is noteworthy that the agreement with temperature and rainfall is closer when one considers the whole of the preceding week than is the case for only the preceding day. The points joined by straight lines on the temperature graph are the mean temperatures for the 24 hours preceding the collecting of the gas sample and the curve thus given does not show the close relation to the carbon dioxide curve that is given by the continuous temperature curve and the same is true of rainfall. Take, for instance, the carbon dioxide value for *B* 20 on 17 April 1935, which is lower than that on 2 April. The temperature for the preceding 24 hours was not lower than that preceding 2 April. But the continuous temperature curve shows a strong dip between the two dates, which might account for the lowered carbon dioxide on 17 April. Again, the lowered carbon dioxide value on 23 July 1935, as compared with that of 9 July, might be explained by the fact that temperatures in the week before 9 July were above that for 8-9 July, while for the week before 23 July they were below that for 22-23 July. This may indicate that the production of carbon dioxide in the soil does not strictly follow the temperature (and possibly rainfall) but that it is a more stable process which only follows the average value; alternatively, it may suggest, and this is perhaps more probable, that the diffusion out of the soil of large quantities of carbon dioxide is a very slow process, so that a gas sample taken at the end of a week will reflect the average rate of production during the week and not the actual rate at the time of taking the sample. Russell & Appleyard (1915) assume that the actual amount of carbon dioxide present at any time represents the rate of production at that time. Their evidence for this is the parallelism between the curves for carbon dioxide and nitrates. Both are produced by similar methods, but nitrates are not lost from the soil by gaseous diffusion. Hence the fact that the variation in amount of both is parallel indicates that the amount present is governed by the production and not by the loss of these substances. There is no means of deciding whether such an assumption would be justified with regard to the Wicken data, since the carbon dioxide curve stands alone. Since the actual amount of carbon dioxide present is so much higher and the soil type so dif-

ferent from those with which Russell & Appleyard worked it seems reasonable to suggest that the percentage of carbon dioxide present at any time might more nearly represent the average rate of production for the preceding few days than the actual rate of production at the time of taking the sample.

(c) *Comparison of the results with those of other investigations*

A large number of results of analyses of the soil atmosphere have been collected together by Romell (1922) and from his study of the data he draws several general conclusions with regard to the percentage of carbon dioxide. In the first place the concentration in any one situation is greater the greater the depth, and secondly the maximum value occurs at the hottest season. The results from Wicken Fen fall in with both these generalizations. On the other hand Romell states that the maximum value is found as a sudden peak in the curve and that most of the values lie near to the minimum. It is true that the curve for *B* 20 is concave upwards for more than half the year but it could not be said to bear out Romell's statement to more than a slight degree.

The greatest interest is perhaps derived by considering the absolute magnitude of the percentages given in Romell's table. Out of 109 values covering a great range of soil type and soil depth, thirty-six of them have a maximum yearly value of less than 1 per cent, thirty-eight have a value between 1 and 3 per cent, twenty-one between 3 and 6 per cent and only thirteen have a maximum of over 6 per cent, so that the soil atmosphere at the locality *B* in Wicken, near to the dyke, may be considered as one of the rarer examples with high values.

The details of these soils with maximum values over 6 per cent have been taken from Romell's paper and set out in Table II. It is noteworthy that except for nos. 12, 13 and 16, the depth of soil in which high values are found is very much greater than anything investigated at Wicken. The three exceptions are from very wet soils and suggest comparable conditions to those in the fen. A point of interest is that the value of 1.8 per cent was found as a maximum at 15 cm. in the Münich *Picea* plantation quoted as no. 8 in the table and the same held good for several of the others, so that in these places the region of soil where roots would develop most abundantly would not show an unusually high carbon dioxide value. It therefore seems as though it is only in situations where the water table is close to the soil surface during the hottest part of the year that plant roots

would be subjected to carbon dioxide values of 6 per cent or over. This conclusion is borne out by Romell's own intensive work on the soil atmosphere in Sweden. The highest values he records are between 5 and 6 per cent and these are invariably associated with a water table close beneath the point from which the sample was taken.

TABLE II. *Carbon dioxide percentages in the soil atmosphere at various places, taken from Romell (1922)*

Locality, type of soil, etc.	Depth below soil surface in dm.	CO ₂ percentage	
		Max.	Min.
1	20	5.8	0.4
2	Dresden, orchard on gravel, water table at 7 m.	6.5	1.4
3		8.1	1.9
4	Klausenburg, Hungary. Grass turf in town.	3.4	0.7
5	Humus rich earth over sand	6.1	1.5
6		14.3	6.9
7	Munich, plot impregnated with sewage	10.6	3.9
8	Munich, plantation, 6-year old <i>Picea</i>	13.3	4.4
9	Munich, plantation, with moss cover	15.2	2.5
10	Munich, plantation, with turf cover	8.3	0.02
11	Munich, plantation, bare	11.6	2.3
12	France, various pasture lands	1.5-6	8.8
13	France, wet lush meadow	2.6.5	11.4
14	Paris, hard-trodden footpaths in parks	4.5-6.0	8.4
15	Pusa, India, manured ricefields bearing crops	20.9	1.4
16	Rothamsted, wet grassland (<i>Festuca ovina</i>)	1.5	9.1

The only results for British soils which are given in Romell's table are those of Russell & Appleyard (1915). These are much more extensive investigations than anything attempted here and involved measurements of bacterial activity and various other soil characters in terms of which the carbon dioxide production might be explained. Their graph for carbon dioxide percentage over two years for the Broadbalk unmanured plot shows a maximum value of 0.8 per cent with most of the values ranging from 0.2 to 0.4 per cent, indicating the altogether different scale of the phenomenon in the two places. Russell & Appleyard obtained a maximum value of 2.2 per cent for a brief period on a manured plot, but for all except waterlogged soils their values were on about the same level as that of the unmanured plot. For waterlogged soil under *Festuca ovina* values from 4 to 9 per cent were found during April and May, but in contrast to the Wicken data the oxygen values fell as low as 2 per cent when carbon dioxide was at a maximum. This rather indicates that it is not sufficient to regard the behaviour of Wicken soils as representing that of other soil

types when they are waterlogged. Rather, the fen peats differ radically, if not in the actual reactions which cause the evolution of carbon dioxide, at any rate in the main factors which affect the speed of the reactions.

The Wicken curves differ also in having a single maximum in July or August, whereas the Rothamsted maximum occurred in spring with a secondary maximum in the autumn. Further, whereas the Rothamsted curves only showed a clear correlation with soil temperature in the winter months, the correlation at Wicken continues throughout. The temperature data for Broadbalk unmanured plot are given as the mean temperature for the 24 hours preceding the carbon dioxide estimation. It has been shown that for the Wicken data this method does not bring out the temperature relationship so clearly as the continuous temperature record. There is no indication in Russell & Appleyard's paper as to whether the same improvement could be effected for their data, at any rate during the winter period when the temperature had a dominating effect over the main drift in carbon dioxide values.

A further difference between the Rothamsted and Wicken results is that in the former there was a strong positive correlation between carbon dioxide percentage and rainfall for the preceding 7 days during the summer, whereas in the latter the correlation is negative. It appears, however, that in some cases at any rate the effect of rainfall at Rothamsted was not immediate, since the carbon dioxide values for the day after rainfall were not appreciably higher, but they rose after 2 or 3 days. Further, since they did not find any very obvious correlation between soil moisture and carbon dioxide percentage, Russell & Appleyard suggest that the action of rain in increasing carbon dioxide output is not due only or mainly to its increasing the soil moisture but to some other influence such as an increased supply of oxygen to soil micro-organisms due to the presence of the gas dissolved in the rain water. Whether this be so or not, there is no doubt as to the main difference of the Wicken soils and the Rothamsted ones and this bears out the idea previously suggested, that the biochemical processes of the fen soils are not comparable except in the very broadest sense with those of mineral soils.

Such a conclusion is, of course, exactly what would be expected by anyone who was familiar with fen peats. There are three main characters of the soil which give rise to this unusual biochemical behaviour. In the first place, the artificial maintenance of the water table at a high level ensures that the soil moisture near the surface is

very high, and yet it is not waterlogged to the exclusion of air spaces. Then, since the soil consists so largely of organic material the amount of carbon which is available for the formation of carbon dioxide is far above that of mineral soils, and lastly the alkalinity of the ground water prevents the development of the acid conditions which generally tend to result from intensive breakdown of plant remains. It is tempting to visualise these conditions as representing the optimum for the majority of bacterial reactions so that their rate is determined almost entirely by temperature. Such a picture is derived only from the one manifestation of carbon dioxide production and it would be of the greatest interest if the investigation could be extended by experts in soil biochemistry.

SUMMARY

1. Continuous records of soil temperatures at Wicken Fen for over 2 years have shown that during this period the temperature in the soil itself has never fallen below 0° C. This is what might be expected from the fact that the temperature diffusivity constant is lower for the fen peat soil (from 0.3×10^{-3} to 2.0×10^{-3} c.g.s. units) than for the mineral soils investigated at Rothamsted (4.0×10^{-3}), and hence the temperature fluctuations in the peat soil are much less. The absence of frost in the soil is a point of significance in relation to the geophytic habit of *Cladum Mariscus*.

2. The carbon dioxide percentages in the soil atmosphere range from 0.5 in the winter to 9.0 in the summer. The general level of the CO₂ curve is very much higher than for most other soils that have been investigated. The shape of the curve differs markedly from that obtained at Rothamsted in that it shows a marked correlation with soil temperature throughout the year, whereas the Rothamsted data only show this correlation during the winter months. Even when the CO₂ percentage is high, the sum of CO₂ per cent plus O₂ per cent does not fall much below 21 per cent in the peat soil, so that unless the soil is actually waterlogged, the O₂ percentage is not likely to fall below 12 per cent.

3. These characteristics of the soil may be related provisionally to the high moisture content of the soil and to the large proportion of the dry weight of the soil which consists of organic material.

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REVIEWS

Practical Problems in Botany. By W. W. ROBBINS and J. ISEN-BARGER. 5 x 8 in. Pp. ix + 402; 230 figures. Chapman and Hall, Ltd. London, 1936. Price 10s.

Although there is general agreement as to the benefits of an inductive method of teaching biology, all teachers recognize the practical difficulties in such a method. It is therefore important to examine carefully any book attempting to teach botany on these lines.

The work under review presents botany as a series of problems: what is protoplasm? where do foods move in the plant? in what ways have plants changed? and so on. The problems are then solved through practical exercises, of which there are 144, and each problem is followed by the theoretical matter which cannot be obtained through observations in class. In this way a comprehensive knowledge of botany should be built round the students' practical work. In another respect the plan of the book is unusual for British readers. There is reference on almost every page to the applications of botany to agriculture, hygiene, and everyday life. This is a consequence of the place botany holds in the United States, a far higher place than it holds in Britain. The importance of botany is due partly, of course, to the greater number of agricultural posts available, but it is also due to the wider recognition in the United States of botany as a cultural subject. The applications to everyday life are sometimes carried to extremes, as for instance in exercise 65, which instructs the student to "devise an experiment using petri dishes of nutrient agar to show that it is not only bad form to sneeze or cough in public without covering the nose and mouth with a handkerchief but that the practice is, besides, decidedly insanitary."

Although the book is an interesting attempt to present botany as a series of problems, it is not a success. It is uncertain for whom it is written, for the text is in that irritating style used in Nature books for small children. Technical terms are sometimes avoided at the cost of accuracy (e.g. "warts" instead of sori, in ferns), and at other times introduced without comment. The practical exercises are not detailed enough to help even the teacher, and that judicious selection of types which is essential to inductive teaching is altogether absent. Exercise 1, for instance, reads as follows: "Make a microscopic study of the different forms of algae which may be collected from ponds, streams, fountains, and moist surfaces of rocks and trees. Observe principally the variations in the form of the plant body. Also, if possible, examine various kinds of brown and red seaweeds." Any teacher knows the confusion which would follow such a stupid procedure.

In addition to these shortcomings the book contains many inaccuracies. The explanation of water absorption by roots on p. 55 is wrong. On p. 75 enzymes are said to be protein in character. One is surprised to find vessels (conducting tubes) labelled in the diagram of a pine stem, redrawn from Strasburger. Reference to the original drawing shows that they are resin ducts.

This book cannot be recommended to British readers, who are already well supplied with accurate elementary text-books. But the "American" course which the authors have tried to follow, of linking up botany with everyday life, and of teaching through practical problems, is one which merits more attention than it has ever received in this country.

E. ASHBY

Mesolithic Settlement of Northern Europe; the Food-gathering Peoples of Northern Europe during the Post-Glacial Period. By J. G. D. CLARK. Pp. xvi + 284, with a map. 4to. Cambridge University Press. 1936. 25s. net.

The circumstances which make it appropriate to review in a botanical journal a book primarily on archaeology rest on two facts: that the physical environment has exercised profound influence upon the development of primitive cultures, and that this environment has undergone very rapid and large changes in the few millenia since the last glaciation. Climatic changes have caused the surface of N.W. Europe to be occupied in turn by fauna and flora of very varying character, and their remains are now available as a natural time-scale for dating and synchronising human cultures. The response to climatic change has been nowhere clearer than in the effect on the major vegetational units of Europe, the forest-belts themselves, which can be demonstrated to have advanced and retreated across the continent in orderly and consistent sequences. Thanks particularly to the technique of pollen-analysis, geological and archaeological phenomena can be related to the phases of forest development surprisingly easily and often, so that investigators in all subjects involving post-glacial history require increasingly precise botanical knowledge about this period.

Foremost among British archaeologists to exploit the new fields of archaeology opened in this way is Dr Grahame Clark. He here extends the scope of his former book—*The Mesolithic Age in Britain*—to cover Northern Europe, which area also chances to be the home of correlated scientific investigation of post-glacial history and the place where most progress has been made in its study.

Dr Clark regards the Mesolithic as a period about 6000 years long in Northern Europe and its cultures as those of food-gathering people living by fowling, hunting, fishing, and collecting. He recognises three main traditions of culture: (i) The tanged point-cultures probably derived directly from upper palaeolithic, (ii) the axe cultures which when influenced by (iii) gave rise to the Maglemose, and (iii) itself, the microlithic or Tardenoisian culture which was introduced into Southern Europe by migration from Afrasia. As chronological basis for study of the development of these cultures the author uses three natural climatic periods:

- I. *Preboreal*—relatively cold—only trees willow, birch, pine.
- II. *Boreal*—rising temperature—at first pine dominant, entry and increase of elm, oak and lime, later of spruce and alder. Hazel often abundant finally.
- III. *Atlantic*—post-glacial warmth maximum—dominance of warmth-loving trees to exclusion of pine and birch except in the far north.

With tremendous energy and skill the author has ransacked the museums and literature of Northern Europe, and has at the same time produced a remarkably integrated study. Whenever possible the implications of pollen-analysis, stratigraphy, climatology and fauna have been brought to bear on the complexities of the cultural developments, and the result is one which no botanist interested in the history of the European flora can afford to neglect. Not only is there a most comprehensive account of all mesolithic sites where pollen-analyses have been made, but in the introductory and final chapters will be found admirable statements of the coordinated changes of natural environment. Extensive appendices and bibliography, clear maps and diagrams and the admirable production make this book the best possible means by which botanists can introduce themselves to the fascinating field of the study of post-glacial history and its many applications to archaeology.

H. GODWIN

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A STUDY OF THE RESPIRATION OF BANANAS

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(With 9 figures in the text)

INTRODUCTION

THE rate of production of carbon dioxide by plant tissues is generally easy to measure and not infrequently is a valuable indicator of changes occurring in the tissues, and a graph showing the course of respiration becomes a datum line to which changes in properties of the tissue can be referred. This is especially true of certain fruits and leaves where, in the course of respiration after gathering, there is a well-defined increase in activity, known as the climacteric rise. The occurrence of such a hump has been reported for apples and pears (Kidd & West, 1930), tomatoes (Gustafson, 1929), bananas (Hartshorn, 1931), and leaves (Godwin & Bishop, 1927).

Its importance in fruit is due to the fact that this increase in the rate of respiration occurs at the time that the fruit starts to ripen.

The respiration of bananas has been measured with fruit of various stages of ripeness under a variety of experimental conditions.

EXPERIMENTAL TECHNIQUE

The two bananas at the outside of a hand were removed and discarded, and the remainder of the hand was then divided up into the required number of samples. The stalks were trimmed with a sharp knife and dusted with boric acid. The samples were stored at a constant temperature, in desiccators or cylindrical jars with glass cover-plates provided with an outlet tube near the top, and an inlet tube reaching to the bottom of the container. Ventilation was obtained by air from cylinders; any traces of carbon dioxide were

removed with moist soda lime. At no time was any evidence obtained of the presence of any foreign vapours or gases in cylinder air which affected the rate of respiration or speed of ripening of the fruit. The rate of air flow was 25 c.c./min. for a container of 6 litres capacity, holding about 500 g. of bananas. The amount of carbon dioxide evolved was estimated by a gravimetric method: moisture was removed by calcium chloride and the carbon dioxide was absorbed by flakes of caustic soda instead of soda asbestos or soda-lime. This method is particularly suitable where the experiment is to run for a long period, and when a large quantity of fruit (up to 1 kg.) is used and the rate of ventilation is slow (50 c.c./min.).

I (a). RESPIRATORY ACTIVITY IN THE PRECLIMACTERIC PHASE

Bananas are transported to Britain as green and unripe fruit. Experimental fruit, generally Gros Michel variety, was obtained from the local depot within 2 or 3 days after being unloaded at Avonmouth. It is not uncommon to find that some of the fruit can be stored for as long as 14 days at 12·5° C. (54·5° F.) before ripening begins. During this time the rate of respiration remains almost steady at a value of 18·5 mg. of carbon dioxide per hour per kg. of bananas. All the samples of green and unripe bananas that were stored at 12·5° C. respired at about this rate; extreme values of 15·0 and 22·35 were occasionally obtained, but did not show any seasonal variation as found by Young *et al.* (1932). It is probable that the rate of respiration during transport remains at a steady value so long as the fruit is unripe and temperature is constant.

By measuring the rate of respiration of green *unripe fruit* at different temperatures it is possible to derive an equation expressing the rate of respiration as a function of temperature. The mean values obtained are given in Table I below.

TABLE I. *Rate of respiration of green bananas at different temperatures*

Temperature	0° C. (32° F.)	5° C. (41° F.)	12·5° C. (54° F.)	15° C. (59° F.)	20° C. (68° F.)	31° C. (88° F.)
Rate of respiration (mg./kg./hr.)	7·1 (7·0)	10·0 (10·3)	18·5 (18·8)	23·6 (23·1)	35·8 (34·6)	61 (84·0)

At 31° C. the rate of production of carbon dioxide in the pre-climacteric phase decreased with time, and at this temperature one might expect the onset of a time factor (Blackman, 1905).

Except for the value at 31°C . (88°F .) these results agree well with the expression $\log R = 0.843 + 0.0348\theta$, where R is the rate of respiration in mg. per kg. per hour and θ is the temperature in $^{\circ}\text{C}$. The values calculated from this expression are shown in parentheses in Table I for comparison with the observed values. The advantage of writing the formula in this form is that the temperature coefficient of respiration, Q_{10} , is readily deduced, since $\log Q_{10} = 10 \times 0.0348$, then $Q_{10} = 2.23$. The rate of respiration is increased nearly $2\frac{1}{4}$ times by raising the temperature 10°C .

Gore (1911) measured the rate of respiration of a large number of fruits at different temperatures, and found that Q_{10} varied from 2.04 to 3.20. Kidd & West (1935) found that Q_{10} for Bramley's Seedling apples was 2.37.

The rate of heat production can be calculated from the above data. If the chemical reaction involved is the oxidation of carbohydrates, then a rate of production of carbon dioxide of 1 mg. per kg. per hour corresponds to a rate of heat production of approximately 10 B.Th.U. per ton per hour. If the values for the rate of respiration in Table I are multiplied by 10, the data then give the rate of heat production in ordinary engineering units.

From the engineering point of view the above results are in error by an unknown amount in that the results refer to detached fruits and not to bunches. When the fruit has reached this country the stalk is readily attacked by micro-organisms. Measurements made with whole bunches, with stalks showing the normal average of post-shipment decay, gave values the same as those stated above for an equal weight of detached fruits.

I (b). THE RIPENING PROCESS AT DIFFERENT TEMPERATURES

The characteristic form of the curve of respiratory activity during the ripening process, at different temperatures, is shown in Fig. 1. Although the preclimacteric value is practically constant at any temperature, the peak value is not so constant.

The effect of temperature on the ripening process as distinct from the time required for ripening to commence can be illustrated by comparing the time taken for the respiration to rise from the pre-ripening phase to the peak value. The times were:

At 12.5°C . (54°F .)	At 15°C . (59°F .)	At 20°C . (68°F .)	At 25°C . (77°F .)	At 31°C . (88°F .)
6 days	4 days	3.2 days	1.5 days	1.5 days

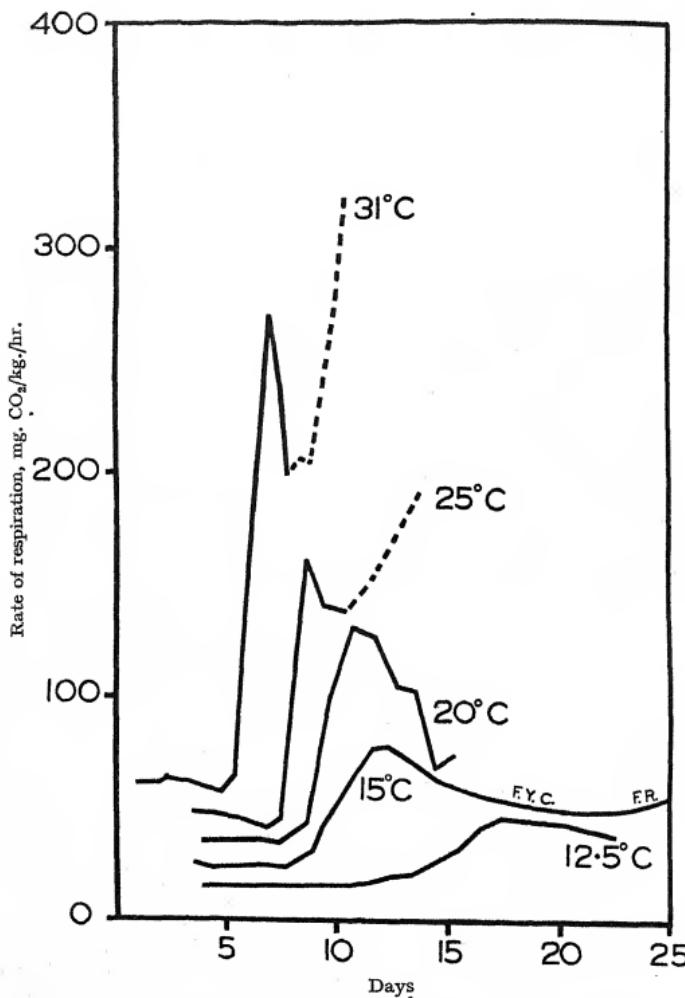


Fig. 1. Rate of production of carbon dioxide by bananas (initially unripe) at different temperatures. The curves at 25 and 31°C. are terminated by the growth of moulds shown by the dotted lines.

With Bramley Seedling apples Kidd & West (1935) found that if t is the duration of the climacteric in days, then $1/t = b\theta$, where θ is in °C. (+3 to 22.5) and b is a constant; this formula, with the same constant, also applies to the climacteric of Conference and Doyenné de Comice pears at temperatures from 1 to 18° C. If the reciprocals of these times are used to express the mean rate of increase of respiration at each temperature, then there is a linear relation between $\log 1/t$ and temperature from 12.5 to 25° C.

The above agree more closely with the formula $\log 1/t = k\theta$ between 12.5 and 25° C.

There is some difficulty in fixing the time at which the climacteric starts, and further work now in progress is directed to start the climacteric by chemical substances at a given time.

The change of colour from green to yellow begins to be visible at the peak of respiratory activity, so that fruit may appear to be green and unripe and yet be well advanced on the climacteric. The climacteric is, however, accompanied by a decrease in the hardness of the fruit, and a "springiness" is detectable soon after the start.

There is one other very marked difference in the course of respiration of bananas as compared with apples and pears. Apples and pears show a continued downward drift in respiration after the peak, which may be slow or rapid, depending on the temperature (Kidd & West, 1935). In bananas the downward drift does not continue, and there is a period when the rate is almost steady; this is followed by a period when it rises again.

These changes in the rate of output of carbon dioxide coincide with the changes in the eating quality of the fruit. Just before the respiration has become steady, the fruit has developed its full yellow colour and the eating quality is at its optimum. From this stage onwards the flavour increases in intensity until finally, at the fully ripe stage, it is too pronounced to be palatable. The relation of these stages to the course of respiration at 15° C. is shown in Fig. 1 where *FYC* refers to the full-yellow-colour stage, and *FR* to the fully ripe stage.

Kidd & West (1935) found that the stage of optimum eating quality of pears occurred at the peak of respiratory activity, and after this there was a rapid deterioration.

It is not possible to define a stage of optimum eating quality of apples except in general terms, and to say that the characteristic flavour develops some time after the peak of respiratory activity and

may persist for a long or short period, depending on the conditions of storage. Most of the apples that are usually eaten have passed the peak of respiratory activity.

I (c). HIGH-TEMPERATURE INJURY

The banana ripens normally in a range of temperature between about 12.5° C. (53° F.) and 30° C. (86° F.). It has been generally found in these experiments with the selected post-shipment fruit used that even a small increase in temperature above these limits caused abnormal ripening. Thus at 31° C. (88° F.) the rate of carbon dioxide production rose to a peak value in the normal manner, but thereafter fell rapidly, and the skin did not develop the full yellow colour of ripe fruit, but was a dull yellow and later developed dark brown areas which became infected by moulds. The pulp was soft and watery. This is a condition known in the Australian markets, when the fruit has been exposed to exceptionally high atmospheric temperatures. Such bananas are said to be "boiled".

Storage in an atmosphere of increased carbon dioxide and reduced oxygen concentrations was found to retard this high-temperature injury to some extent; for example, in an atmosphere containing 10.5 per cent of carbon dioxide and 9.5 per cent of oxygen (an atmosphere obtained by ventilating with a gas-mixture, from a cylinder, of 10 per cent carbon dioxide, 10 per cent oxygen, and 80 per cent nitrogen), bananas were stored at 31° C. (88° F.) for 7 days, and then ripened normally in air at 15° C. (59° F.). After storage at 31° C. (88° F.) for 10 days, however, even in this atmosphere, the characteristic "boiled" appearance was observed. The possibility of transporting bananas through unavoidably high atmospheric temperatures with the aid of restricted ventilation and reduced oxygen concentrations may, however, be worth further study.

I (d). RESPIRATION AT LOW TEMPERATURES

Fig. 2 shows the effect of transferring bananas to various lower temperatures, at a stage when they had reached the peak of their respiratory activity at 12.5° C. (54° F.), and had visibly commenced to ripen. The temperatures used were 0° C. (32° F.), 5° C. (41° F.), and 10° C. (50° F.). A control sample was kept at 12.5° C. (54° F.) throughout.

At 5° F. the respiratory activity was depressed, but when this fruit was transferred back to 54° F. after 14 days, the activity

characteristic of the higher temperature was restored. At 41° F. the respiratory activity was still further depressed, but was again restored on transference back to 54° F. after 12.5 days.

At 32° F. the curve of respiratory activity appeared to consist of two portions. There was a steep fall to a level lower than that obtained at 41° F., followed by a steady period similar to that shown at 41 and 54° F. After 5 days, however, there was a further slow but continuous fall, apparently towards a zero rate of respiration. After 15.5 days these bananas were transferred back to 54° F. The respiratory activity increased temporarily, and then fell off practically to zero.

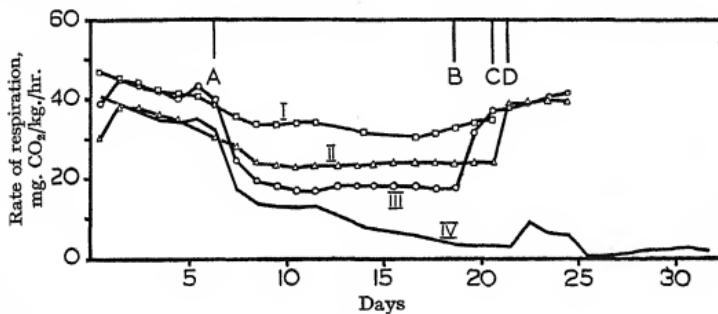


Fig. 2. Rate of production of carbon dioxide by ripe bananas at different temperatures. Bananas I were control, maintained at 12.5° C. throughout. At A, bananas II, III and IV were transferred to 10, 5 and 0° C. respectively; at B, bananas III were returned to 12.5° C.; at C, bananas II were returned to 12.5° C.; at D, bananas IV were returned to 12.5° C.

The data suggest that the depression of respiratory activity at low temperature is at first reversible, but that irreversible effects slowly supervene, and cause a progressive lamming of the respiratory mechanism, until finally the fruit is killed.

Alcohol and acetaldehyde were found in the tissues of the bananas exposed for a long period to 0° C.

The rates of respiration during the steady period were:

12.5° C.	10° C.	5° C.	0° C.
33.4	22.9	17.9	12.9

These results agreed fairly well with the expression

$$\log R = 1.096 + 0.0334\theta,$$

where R is the rate of respiration in mg. per kg. per hour and θ is the temperature in ° C. The equation is of the same form as that given

under I (*a*) for preclimacteric fruit. The temperature coefficient is the same in both cases. The constants of the two equations show that at the same temperature the rates of respiration of unripe fruit and fruit which has developed its full yellow colour are in the ratio of 1 : 1.8.

I (c). EFFECTS OF TEMPORARY EXPOSURE TO LOW TEMPERATURES

These observations were extended by investigating the effect of temporary storage at 0° C. (32° F.) at various stages of ripening. Fig. 3 summarizes these results. Bananas were transferred from 12.5° C. (54° F.) to 0° C. (32° F.) at four stages of maturity, namely:

(*a*) Just at the commencement of the rise in respiratory activity (after 4 days at 12.5° C. (54° F.)).

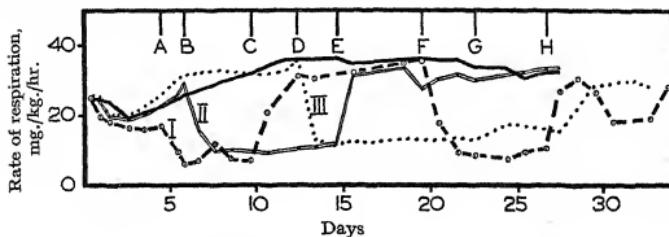


Fig. 3. Rate of production of carbon dioxide by bananas at 12.5 and 0° C. At A, bananas I were transferred to 0° C.; at B, bananas II were transferred to 0° C.; at C, bananas I were returned to 12.5° C.; at D, bananas III were transferred to 0° C.; at E, bananas II were returned to 12.5° C.; at F, bananas I were transferred to 0° C.; at G, bananas III were returned to 12.5° C.; at H, bananas I were returned to 12.5° C. One sample shown by continuous thick line was kept at 12.5° C. throughout.

(*b*) Near the peak of respiratory activity (after 6 days at 12.5° C. (54° F.)).

(*c*) Just beyond the peak of respiratory activity (after 12 days at 12.5° C. (54° F.)).

(*d*) Well beyond the peak of respiratory activity (after 20 days at 12.5° C. (54° F.)).

Lot (*a*) were stored at 0° C. (32° F.) for 5 days, lot (*b*) for 8 days, lot (*c*) for 9 days, lot (*d*) for 6.5 days.

In all cases the rate of carbon dioxide output fell to a steady value at 0° C., and in all cases it rose again to the level of the controls when the bananas were returned to 12.5° C. The fruits were analysed at the end of the experiment, and did not then show any pronounced accumulation of alcohol or acetaldehyde.

The extent to which the changes in respiratory activity are reversible is more than a little surprising, in view of the pronounced susceptibility of green fruit to damage by "chilling" during shipment. There are two possibilities—one that the respiratory activity is not an accurate index of the damage, the other that bananas such as were used in this experiment, the most immature being only 2 days from the beginning of the climacteric, are not so susceptible as immature fruit.

In the present case the fruit subjected to low temperature was not blackened. It appeared to ripen normally, though critical examination from this point of view was not possible with the small number of fruits employed.

I (f). PRODUCTION OF CARBON DIOXIDE IN NITROGEN

When unripe bananas at 15° C. (59° F.) were transferred from air to nitrogen the rate of carbon dioxide production remained steady for 2 days, and then decreased slowly to a value which was almost zero

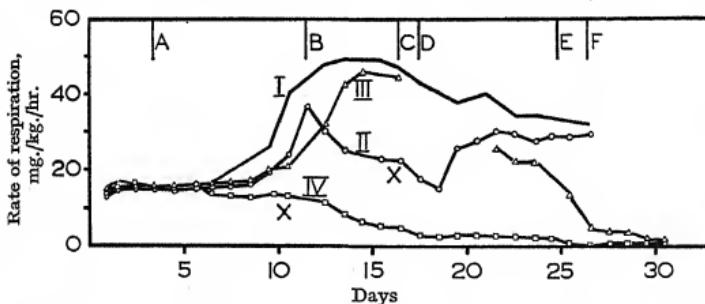


Fig. 4. Aerobic and anaerobic production of carbon dioxide by bananas at 15° C. At A, bananas IV were transferred from air to nitrogen; at B, bananas II were transferred from air to nitrogen; at C, bananas III were transferred from air to nitrogen; at D, bananas II were returned to air; at E, bananas III were returned to air; at F, bananas IV were returned to air. Bananas I were stored in air throughout.

after 24 days. Even after it was returned to air this fruit remained hard and green, and its carbon dioxide production did not increase.

When the transfer from air to nitrogen was made during the rapid rise of carbon dioxide production characteristic of ripening, or after the peak value, there was a rapid decrease in the rate. The new level was not, however, reached as quickly as when similar bananas were transferred to a low temperature. The data are given in Fig. 4.

The effects of transference from nitrogen back to air were variable, and further experiments are proceeding.

The view held at present on the basis of the results here reported is that in the banana the ratio of carbon dioxide production in air and in nitrogen (*OR/NR* ratio of Blackman (1928)) is not far from unity: that a process of slow metabolic adjustment then takes place in the absence of oxygen, leading to a new equilibrium in which the carbon dioxide production in nitrogen is less than in air (0·6–0·8). Damaging effects then supervene, causing a fall off in activity and loss of the power of recovery on return to air. The inflexion points marked (X) suggest the time at which these damaging effects begin.

I (g). RESPIRATION IN ATMOSPHERES CONTAINING CARBON DIOXIDE

Very little investigation of the effects upon respiration of atmospheres containing considerable quantities of carbon dioxide has been carried out, owing largely to the difficulty of measuring accurately the addition of a small amount of carbon dioxide to a current of air already rich in this gas.

Method. The katharometer has been used as the measuring instrument for carbon dioxide in the present research in a way which, as far as we know, is novel, and has proved very simple in manipulation, especially if an increase of 1 per cent can be tolerated between the ingoing and outgoing current of air through the respiration chamber.

A differential meter was used, and the gas stream was passed over one limb, then through the chamber containing the bananas, and finally back over the second limb. A short-circuiting device enabled the zero of the instrument to be checked whenever desired, without altering the rate of flow of the gas through either limb, or through the container. The relative humidity should, of course, be kept the same on both sides of the container, and the rate of the air current must be measured.

The apparatus is shown diagrammatically in Fig. 5.

The gas mixture, 10 per cent oxygen, 10 per cent carbon dioxide and 80 per cent nitrogen, was obtained in steel cylinders. Periodic tests have shown that the composition of the gas from the cylinder is practically constant during the whole period of discharge.

The gas stream was saturated by bubbling the air through a filter candle immersed in water. The current through the katharometer was kept steady at 120 ± 0.1 mA. The katharometer bridge was put

slightly out of balance, and then adjusted by a large shunt (755 ohms) across one arm. With the particular instrument used the shunt was altered by 15·6 ohms when there is a difference of 1 per cent of carbon dioxide in the air in the two chambers. The rate of air flow was

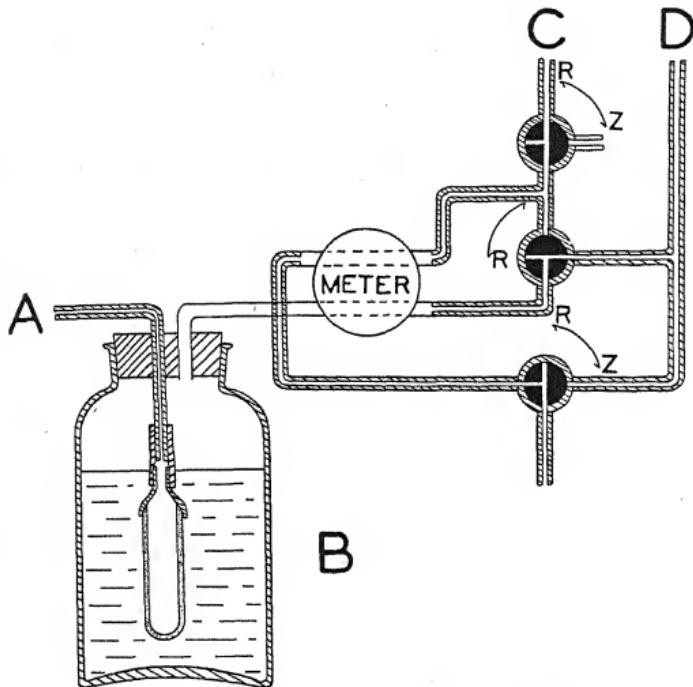


Fig. 5. Diagram of apparatus used to measure the respiration of bananas in gas mixtures. A, connected to gas mixture in steel cylinder; B, wash bottle to control humidity; C, connected to outlet tube of fruit container; D, connected to inlet tube of fruit container. With the three three-way taps in the positions illustrated, the change in composition of the gas mixture can be measured by the meter. The three taps are turned through 90° in a clockwise direction to check the zero of the meter.

measured by a calibrated flowmeter and checked each day by collecting a sample over water.

Results. When green bananas were transferred from air to an atmosphere of 10 per cent carbon dioxide, 10 per cent oxygen and 80 per cent nitrogen, the rate of production of carbon dioxide was reduced at once to about three-fourths of its value, and from this

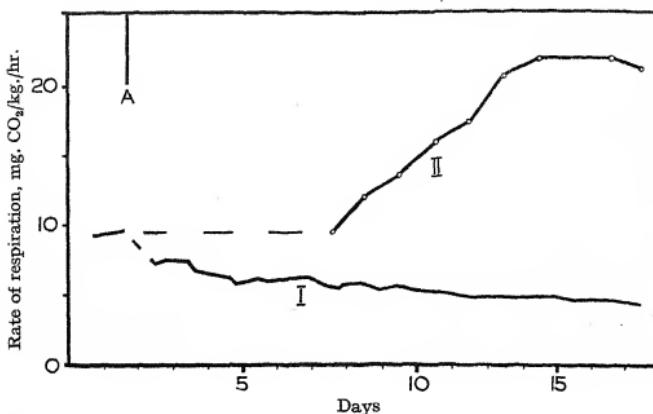


Fig. 6. Rate of production of carbon dioxide by unripe bananas in an atmosphere of 10 per cent carbon dioxide, 10 per cent oxygen, 80 per cent nitrogen (15°C). Bananas II were stored in air throughout; at A, bananas I were transferred to gas mixture.

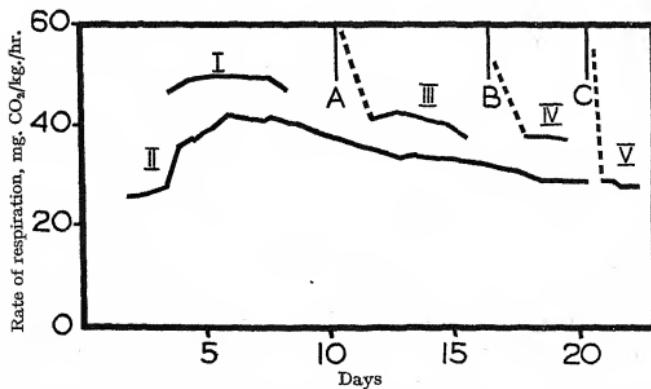


Fig. 7. Rate of production of carbon dioxide by bananas in an atmosphere of 10 per cent carbon dioxide, 10 per cent oxygen, 80 per cent nitrogen (15°C). Bananas I in air. Bananas II in gas mixture. At A, bananas III transferred to air; at B, bananas IV transferred to air; at C, bananas V transferred to air.

point fell off slowly and steadily for 14 days (Fig. 6A). There was no peak of respiratory activity, and the fruit did not ripen. The control fruit behaved normally.

When the experiment was repeated with a number of bananas in the container, and probably at a rather more advanced stage of maturity, the fruits ripened, and there was a peak of metabolic activity, but at a value about 20 per cent lower than that for similar bananas in air (Fig. 7).

When individual bananas were taken back to air at various stages in the course of ripening, the rate of output of carbon dioxide rose to a new value, about 20 per cent higher than that in the gas mixture. There was, of course, a large transitional effect due to the liberation of absorbed carbon dioxide from the tissues.

The effects of 10 per cent carbon dioxide on the banana are (*a*) the suppression of the climacteric, and (*b*) the reduction of respiratory activity in both the preclimacteric and postclimacteric phases, and are thus qualitatively the same as the effects of 10 per cent carbon dioxide on apples, as has been shown previously in this laboratory (Kidd & West, 1934).

II (a). RESPIRATION AND CARBOHYDRATE CHANGES

The rapid changes in the carbohydrates in the pulp of bananas during ripening have been previously examined (Bridel & Bourdouli, 1929; Stratton & Loescke, 1930). At the temperature of ripening rooms (68–70° F.) in the comparatively short period (10 days) the amount of starch in the pulp falls from about 20 to 2 per cent, with a corresponding increase in the quantity of soluble sugars. These changes have not been correlated with the course of respiratory activity.

One experiment was started with one hand of green unripe fruit, and all the fruits of the hand were numbered and weighed separately. The respiration of all the fruits together was measured at 15° C. Each day one fruit was removed from the container, peeled and the pulp weighed. This was stored in stoppered jars at –20° C. until the end of the experiment. The pulp was dropped into boiling alcohol, cut into thin slices and extracted in a soxhlet extractor. The extract was treated in the usual way, clearing being done with lead acetate, followed by sodium phosphate as a deleading agent.

The data are given in Table II.

The respiration rates given in Table II are the mean values for periods of 24 hours, and the sugar values are those of the pulp at the

TABLE II. *Carbohydrate changes in the pulp of bananas during the early stages of ripening*

Day	Respiration mg./kg./hr.	Sugar content (g. per 100 g. fresh weight)	
		Sucrose	Reducing sugars
1	—	0.572	0.171
2	23.4	0.659	0.251
3	22.6	0.642	0.217
4	21.7	0.608	0.236
5	21.2	0.637	0.291
6	23.7	0.707	0.310
7	24.1	0.874	0.606
8	25.2	0.804	0.432
9	32.5	0.922	0.621
10	40.2	1.050	0.593
11	52.0	2.636	1.125
12	62.2	4.076	1.361
13	73.0	5.764	1.738

end of each period of 24 hours. The mean rate of respiration is compared with the *mean* sugar content for each period and the data are plotted in Fig. 8.

There is obviously a very close connexion between the increase in the rate of respiration during the climacteric and the amount of soluble sugars in the pulp. Barker (1933, 1936) has examined the relation of the respiration of potatoes to the concentration of sugars, and found that "the respiration/sucrose relation conformed closely with the enzymatic rectangular hyperbola for the rate of reaction/substrate relation for an enzyme reaction *in vitro*".

The R /sucrose and R /hexose relations in Fig. 8 clearly demonstrate a close connexion between the increase in rate of respiration during the climacteric and the amount of soluble sugars in the pulps. To ascertain how clearly the curves conform with the enzymatic rectangular hyperbola, the reciprocals of the values of respiration and the sugars are plotted in Fig. 8. If the relation between R and either sucrose or the hexoses is expressed by a rectangular hyperbola, this plotting should give a straight line.

The $1/R/1$ /sucrose relation does not depart far from linearity, while the relation with the hexoses shows marked departure. So far then as may be judged from this single set of data for the relation between R and sugars during the climacteric rise in R , the rate of R appears to be more closely related to the concentration of sucrose than to the hexoses.

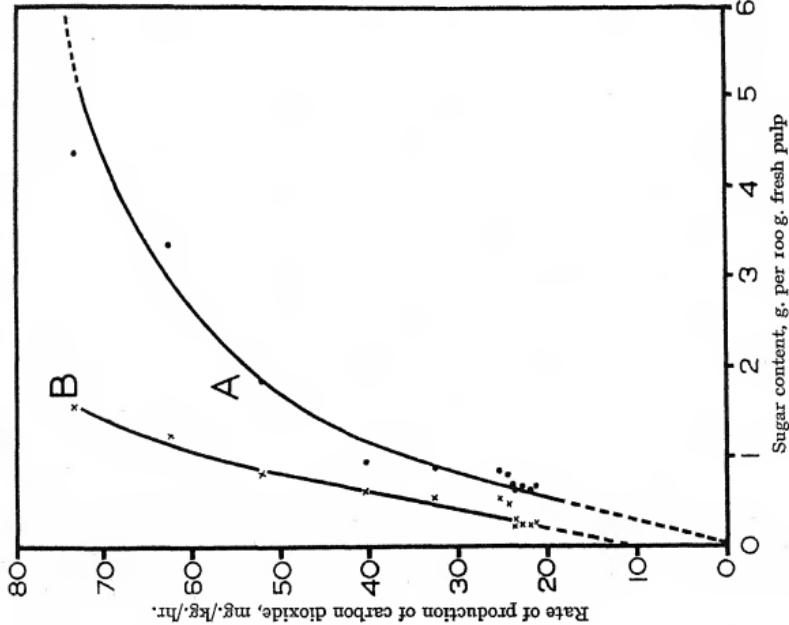
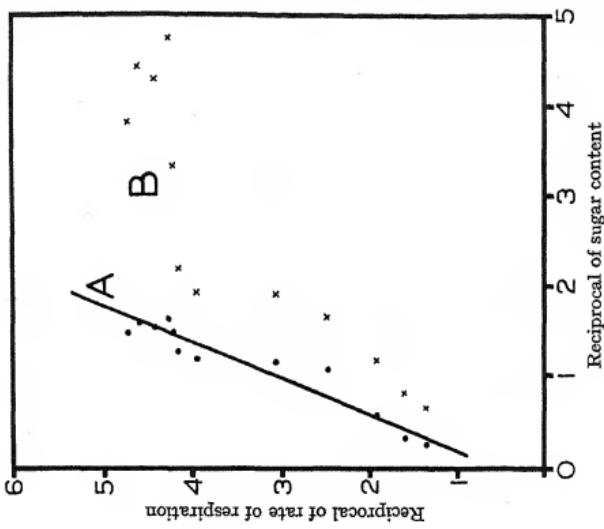


Fig. 8. Rate of production of carbon dioxide by bananas, and the sugar content of the pulp. A, sucrose; B, reducing sugars.

The data given here only cover the period in which the rate of respiration changes rapidly and reaches its maximum value. After the peak value of respiration, the sugar content of the pulp continues to increase, and it is clear that factors other than sucrose limit the rate of respiration.

II (b). pH OF THE PULP

The pH of the pulp has been measured, using a glass electrode with a quadrant electrometer detector sensitive to $\pm 0.01 \text{ pH}$. A number of fruits from one hand were stored in a desiccator and the respiration measured. Single fruits were removed at intervals,

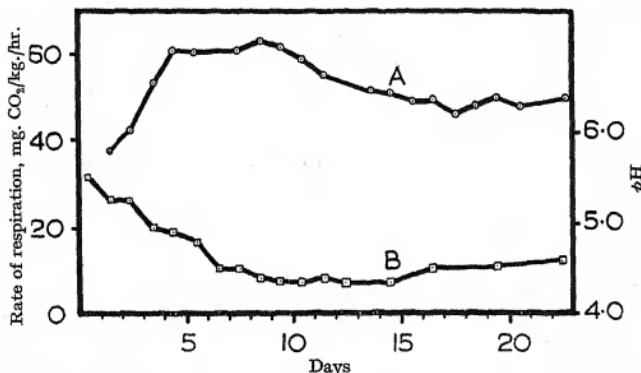


Fig. 9. Rate of production of carbon dioxide by bananas, and pH of the pulp (15°C). A, rate of production of carbon dioxide; B, pH of pulp.

peeled and the pulp crushed in a porcelain mortar. The expressed fluid of the unripe fruit or the macerated pulp of ripe fruit was placed in the cup of the glass electrode.

The pH of the pulp of bananas in the preclimacteric phase was found to be approximately constant at 5.8. A fall in pH was coincident with the climacteric rise in respiratory activity, and continued until a minimum value of 4.3 was reached some time after the peak of respiratory activity. There was a slow upward drift of pH when the respiration increased again. The data are shown in Fig. 9. Further examination is in progress and consists in the measurement of the titration/ pH relations of the pulp.

Plagge & Gerhardt (1930) found that in Jonathan apples the pH fell from 3.01 to 2.87 in the first 12 weeks of storage at 36°F ., and then

rose to 3.18 after 27 weeks' storage. Data given by Haynes & Brown (1928) show that there was a slight increase in *pH* in Lane's Prince Albert and Cox's Orange Pippin apples in the first four months of storage, after which it did not change very much.

II (c). VOLATILE SUBSTANCES PRODUCED DURING AND AFTER RIPENING

An estimate of the odorous substances produced by the fruit can be obtained by measuring the total combustible matter in the air after it has passed over the fruit. This is easily carried out at the same time as measurements are made of respiration. After the carbon dioxide has been removed by a weighed tube of flaked caustic soda, the air was then passed through a tube heated to 800° C. and containing

TABLE III. *Total combustible gaseous products of bananas during ripening at 15° C.*

Days	Experiment I		Experiment II		Experiment III	
	Respira- tion (carbon dioxide in mg./ kg./hr.)	Vapour (as carbon dioxide in mg./ kg./hr.)	Respira- tion (carbon dioxide in mg./ kg./hr.)	Vapour (as carbon dioxide in mg./ kg./hr.)	Respira- tion (carbon dioxide in mg./ kg./hr.)	Vapour (as carbon dioxide in mg./ kg./hr.)
1	—	—	—	—	45.3	0.08
2	30.2	0.17	—	—	44.1	0.08
3	32.8	0.13	32.4	0.11	43.6	0.07
4	39.6	0.09	46.5	0.12	57.1	0.06
5	51.5	0.11	53.1	0.07	71.6	0.05
6	59.0	0.10	61.6	0.16	75.0	0.04
7	56.1	0.08	70.2	0.07	77.0	0.04
8	50.8	0.08	66.9	0.09	76.0	0.04
9	50.1	0.07	69.3	0.03	72.7	0.04
10	50.1	0.08	69.6	0.04	62.9	0.03
11	50.2	0.06	65.7	0.03	58.6	0.04
12	53.2	0.07	61.7	0.01	53.6	0.02
13	52.4	0.06	59.3	0.08	47.3	0.03
14	51.2	0.06	56.7	0.04	Ventilated with oxygen	
15	—	—	53.9	0.04		
16	55.2	0.03	55.5	0.02		
17	53.0	0.03	55.8	0.04		
18	52.9	0.01	51.2	0.02		
19	53.6	0.01	53.8	0.13		
20	54.9	0.03	55.3	0.10		
21	55.3	0.01	57.7	0.10		
22	—	—	59.0	0.67		
23	—	—	61.1	0.88		
24	—	—	65.0	—		
25	—	—	64.2	1.81		
26	—	—	64.3	1.58		
27	—	—	65.5	—		

copper oxide. The organic substances in the air were burnt to water and carbon dioxide, and the latter was then absorbed in a standard solution of caustic soda in a pettenkofer tube.

It is well known that it is difficult to burn completely small quantities of organic vapours present in very low concentrations in air. In these experiments the rate of ventilation and therefore rate of passage of the gas through the furnace tube was limited by the rate at which air could be passed through a pettenkofer tube. The rate did not exceed 30 c.c. per min. so that, with the furnace tube used, the time available for combustion was of the order of 1 min. This is considerably faster than the rates used in a combustion furnace in the determination of carbon and hydrogen in a solid or liquid, but not faster than the rates used to burn combustible gases, e.g. coal gas mixed with air.

The amount of carbon dioxide so obtained was very small, and appreciable amounts were not obtained until long after the full yellow colour of the fruit had developed and the respiration had started to increase again. The fruit was then very soft and susceptible to mechanical injury, and had a very strong flavour. The data are given in Table III.

The effects on the banana of one of the constituents of the volatile substances and of ethylene will be considered in a later paper.

SUMMARY

1. The respiration of unripe bananas has been measured at temperatures of 12·5, 15, 20, 31 and 32° C. The values lie near a smooth curve for which a mathematical expression has been found:

$$\log R = 0.843 + 0.0348\theta.$$

The temperature coefficient of respiration Q_{10} , between 12·5 and 20° C., is 2·23.

2. The time of duration of the climacteric is an exponential function of temperature.

3. Injury to the fruit occurs at high temperatures (31° C. and above) and may be prevented if the exposure is not too long by storing in an atmosphere with an increased concentration of carbon dioxide.

4. Short exposures of the fruit to low temperatures quickly reduce the rate of respiration to a lower level which is then maintained during the exposure: when returned to the higher temperature, recovery is rapid and complete.

5. The anaerobic production of carbon dioxide by bananas at 15° C. varied with the degree of maturity of the fruit. In preclimacteric fruit the anaerobic production of carbon dioxide is the same as the aerobic for a short period, after which there is a steady fall, which in 20 days reaches almost zero. At or after the peak of aerobic activity, the anaerobic rate is much lower than the aerobic rate and falls steadily.

6. The respiration in a gas mixture consisting of 10 per cent oxygen, 10 per cent carbon dioxide and 80 per cent nitrogen is reduced to 80 per cent of the value in air; with one sample under these conditions the climacteric was delayed and did not occur within the period of the observations.

7. Respiration during the climacteric is related to the concentration in the pulp of total soluble sugars and to sucrose. The relation between them is expressed by a rectangular hyperbola.

8. The pH of the pulp decreases during and after the climacteric, and reaches a minimum value when the fruit has developed its full yellow colour. After this stage there is a slow increase.

9. The total volatile substances produced by the fruit have been determined as carbon dioxide by combustion. The quantities obtained were extremely small until the fruit reached an "over-ripe" stage.

The author wishes to express his thanks to Dr F. Kidd for his invaluable help and criticism. He is indebted to Miss D. G. Griffiths and Miss N. A. Potter for the carbohydrate data presented in Table III.

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THE EFFECT OF POTASSIUM SUPPLY ON THE WATER RELATIONS OF FOLIAGE LEAVES

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(With 4 figures in the text)

THE complexity of the relation between potassium supply and the water content of foliage leaves is well illustrated by the conflicting results which have, from time to time, been reported. With a variety of fruit plants Wallace (1931) finds that potassium deficiency is accompanied by a lowered water content in the foliage. Mann (1924) reports essentially similar results, also for fruit trees. With the potato James (1930) finds an agreement between the spatial distribution of potash and of water within the plant, but obtains an increased water content in the foliage with an increased potash supply only when the additional potash is supplied as chloride, and ascribes the effect to the chloride ion. Gregory & Richards (1929), in water culture experiments with barley, obtained with increased potash supply a decreased water content in the foliage. Manifestly, however, when the results are expressed on either the conventional fresh-weight or dry-weight basis, apparent variations in water content may be due as much to variations in dry matter as to real variation in water content. Certainly, a deficiency of potash is with a wide variety of plants reflected in the withering and scorching of the foliage (Wallace, 1934), and the assumption has been that the withering is the result of unfavourable conditions of water supply within the plant (Summers, 1922; Wallace, 1928).

The results which will be described here were obtained from an experiment set up in 1933, in order to attempt an elucidation of the nature of the relation between potash supply and the water balance in foliage leaves.

EXPERIMENTAL

Material

Foliage of seakale beet (*Beta cicla*) constituted the bulk of the material utilized, but water cultures of buckwheat were also used. Three sets of seakale beet were available which may conveniently be designated (a) garden plots, (b) soil pots, and (c) sand pots respectively.

(a) Experimental plots were laid down in the gardens, the soil of which is a heavy loam which had in previous years been heavily manured with organic manures. The soil of the plot is rather low in available potash, especially when this is considered in relation to the "available" phosphate content as the following analytical figures demonstrate: "available" potash (K_2O), 0.011 per cent; "available" phosphate (P_2O_5), 0.067 per cent, of air-dry soil.

Three treatments were given and each treatment duplicated so that a total of six plots was laid down as follows: All the plots received a dressing of ammonium phosphate at the rate of 1 cwt. per acre. Two plots received no additional potash, two received potassium sulphate at the rate of 2 cwt. per acre and two received potassium chloride at a rate sufficient to supply the same amount of potash (K_2O) as the potassium sulphate plots received. The manures were applied during July and seed sown on 26 July in drills 1 ft. apart and the plants singled to 1 ft. in August. Sampling commenced in October, continued until the end of November and was recommenced in April and continued until June.

(b) *Soil pots.* For these plants soil from an area known to be deficient in potash was used, and which on analysis gave "available" potash 0.007 per cent, and "available" phosphate 0.028 per cent of air-dry soil. The plants were grown singly in 5 in. pots. Each pot received 0.5 g. ammonium phosphate. Twenty-five pots received no additional potash (series G), twenty-five received 2 g. of potassium sulphate (series H) and twenty-five an amount of potassium chloride equivalent to 2 g. of potassium sulphate (series I). Seed was sown on 1 August and the plants taken indoors in October.

(c) *Sand pots.* Three series of plants were grown in 5 in. waxed pots containing silver sand and supplied with nutrient solution. All the solutions used contained phosphate (P_2O_5) 284, nitrogen 140, lime (CaO) 112, and magnesia (MgO) 80 parts per million. The solution supplied to plants of series J contained no potash, that supplied to series K 132 parts, and that supplied to series L 658 parts of potash (K_2O) per million. These plants grown in pots were used only for experiments on transpiration and stomatal movement.

Buckwheat (Polygonum Fagopyrum Linn.)

Three sets of buckwheat plants were grown in water culture, the culture solutions used being identical in composition with those used for the sand cultures of the beet.

RESULTS

The results can be conveniently presented in two sections referring respectively to (1) outdoor plots, and (2) experiments on transpiration and stomatal movement.

Section I

Throughout this section it will be convenient to designate the three treatments by the terms no potash, chloride and sulphate, indicating the plants receiving no additional potash, those receiving potassium chloride and those receiving potassium sulphate respectively.

Determinations were made of leaf area, leaf number, stomatal frequency, area of epidermal cells, water content, dry-matter content, water of "imbibition", water of imbibition after leaching, residual water, contents of potash, sulphate and chloride, and osmotic pressure of the expressed sap. Data for water content were collected during both sampling periods, and the two sets of results are presented. The main measurements of leaf size refer to large samples measured on one date. Further smaller samples were measured in connexion with the osmotic pressure determinations and the measurements of cell size, and these data will also be presented.

The data collected are assembled in Table I. The significance of the treatments was examined, usually by means of Fisher's Z test (1930), and the values of Z between Treatment and Remainder are entered in the table, together with the values of Z for a 5 per cent probability. When all the determinations were made on a single occasion, the standard errors of the mean values were calculated and these are attached to the mean values in the table.

Following the table, the experimental methods and the results are discussed *seriatim*.

Experimental methods and results

(1) *Leaf size.* The main set of measurements of leaf size were made on 8 November 1934, the length and width of the lamina being determined for each leaf. For each treatment about two hundred leaves were measured, and it is seen that the additional potash whether supplied as chloride or sulphate has effected a significant increase in leaf size.

(2) *Number of leaves per plant.* Counts of the number of mature leaves per plant were made on samples of approximately eighty plants, and the plants receiving the additional potash supply are seen to have an increased number of leaves.

TABLE I

	Leaf size		No. of leaves per plant	No. of stomata per mm. ² of leaf
	Length cm.	Width cm.		
No potash	22.04 ± 0.25	14.92 ± 0.20	2.96 ± 0.118	158 ± 7.7
Chloride	25.90 ± 0.27	16.66 ± 0.17	3.41 ± 0.130	121 ± 6.2
Sulphate	26.90 ± 0.26	17.03 ± 0.26	3.40 ± 0.133	125 ± 7.4

October–November 1933

	Water content			Dry matter g. per 100 cm. ² of leaf	Residual water g. as % dry weight
	As % dry weight	g. of water per 100 cm. ² of leaf	g. per 100 cm. ² of leaf		
No potash	723.9	3.13	0.4335	6.97	
Chloride	787.9	3.32	0.4232	9.51	
Sulphate	801.7	3.42	0.4419	12.15	
Z treatment remainder	1.0972	0.9070	—	—	
Z for 5% point	0.6451	0.6451	—	—	

April–June 1934

	Water content		Dry matter	Water of "imbibition" after leaching
	As % dry wt.	g. per 100 cm. ² leaf	g. per 100 cm. ² leaf	% dry wt. / % dry wt.
No potash	621.2	2.80	0.450	20.52 / 11.34
Chloride	649.8	2.94	0.453	21.84 / 11.35
Sulphate	623.8	3.09	0.495	21.85 / 11.36
Z treatment remainder	0.9448	0.6702	1.1922	1.0383 / —
Z for 5% point	0.7058	0.7058	0.7058	0.7058 / —
	Potash (K ₂ O) % dry wt.	Chloride (Cl) % dry wt.	Sulphate (SO ₄) % dry wt.	
No potash	1.51	0.21	1.57	
Chloride	2.07	0.39	1.49	
Sulphate	2.68	0.23	1.78	
Z treatment remainder	1.5961	1.2110	1.2380	
Z for 5% point	0.6594	0.6594	0.6594	

	Mean area of epidermal cells mm. ²		Mean area of leaf cm. ²	Freezing point of expressed sap (° C.)	Ultimate leaf area cm. ²
	Mean area of epidermal cells mm. ²	Mean area of leaf cm. ²			
No potash	0.00105 ± 0.00007	1.88 ± 14	—	— 0.999	158
Chloride	0.00145 ± 0.00013	2.83 ± 28	—	— 1.033	209
Sulphate	0.00163 ± 0.00009	3.04 ± 43	—	— 1.058	189
Z treatment remainder	—	—	—	— 0.3190	1.2527
Z for 5% point	—	—	—	— 0.7058	0.7058

(3) *Stomatal frequency.* Stomata are present in equal numbers on both sides of the leaf of the seakale beet, but all counts refer to the upper surface. In order to eliminate as far as possible errors due to the varying intensity of stomatal frequency in different regions of the leaf, strips of epidermis were always taken from midway between two main lateral veins and about one-third of the length of the leaf blade from the apex of the leaf.

The additional potash has effected a significant decrease in the number of stomata per sq. mm. of leaf surface. Between treatments the number of stomata per unit of leaf surface is clearly inversely related to leaf size. There is here a clear indication that increase in leaf size has been effected through an increase in cell size, resulting in a wider spacing of the stomata. If this is so it means that the effect of the increased potash supply is operative during the period of cell extension. Salisbury (1927) has previously pointed out that, whilst stomatal frequency varies, the variation is due mainly to a difference in the size of cells rather than to a difference in the stomatal index.

(4) *Cell size and leaf size.* It appeared likely from the determination of stomatal frequency and leaf size that the increased potash supply had effected an increase in leaf size through its effect on cell size. In order to test this hypothesis measurements of cell size were made together with determination of the area of the leaves used. For this purpose, strips of epidermis were taken and examined microscopically at once and the number of epidermal cells falling within a square of known area on the field counted. Each mean value given in the table is the mean of approximately two thousand cells. It is apparent from the table that the increase in leaf size brought about by the increase in the potash supply is accompanied by a proportionate increase in the size of individual cells.¹

(5) *Water and dry-matter contents.* For the purpose of estimating water content, segments of known area were cut, one on either side of the midrib, midway between the edge of the leaf and the midrib. The method of sampling is important as the water content decreases in passing from the midrib region to the edge. As it was necessary to sample only when the leaves were free from extraneous moisture, sampling was carried out at somewhat irregular intervals. The leaves were always gathered between 2 and 2.30 p.m., and only fully expanded leaves showing no sign of yellowing were used. Water content was determined by drying for 24 hours at 103° C. As segments of known

¹ A similar set of measurements on the buckwheat plants confirm this.

area were used, it is possible to express both water and dry-matter content on an area basis.

For the autumn period of sampling the results are quite clear. The treatments involving an increase in the supply of potash have given an increase in the water content, expressed as a percentage of dry weight. Both treatments give a significant increase, whilst the difference between the chloride and sulphate treatments fails to reach the level of significance. A similar result is obtained when the water content is expressed on an area basis. For the spring sampling period only the chloride treatment gives a significant increase in the water content expressed as a percentage of dry weight. The sulphate treatment, however, gives here an increase in the amount of dry matter per unit area of leaf, and this it appears masks an increased water content. It is almost certain, therefore, that for this sampling period, too, both treatments have given a real increase in the water content of the leaves.

(6) *Residual water.* This term is used here to define the amount of water retained by the leaf material after exposure to a desiccating influence of definite intensity for a definite time. After various trials leaf segments were suspended over calcium chloride at 32° C. for 18 hours, and the amount of water retained at the end of this period determined by drying to constant weight at 103° C. Both the chloride and sulphate treatments are seen to have increased the amount of residual water. The increase, however, runs parallel with increases in the potash content of the material used. There is therefore a suggestion that the differences observed are due to the varying amounts of hygroscopic potash salts present rather than to differences in colloid swelling.

(7) *Water of imbibition.* The term "water of imbibition" is used here to define the amount of water which dried leaf material will imbibe when exposed under conditions of definite humidity and temperature. Determinations of this nature were made by Pearsall & Ewing (1929) in their studies on succulence, and the data were considered to represent the affinity for water of the colloidal material of the plant tissue. In this experiment leaf segments were dried over calcium chloride at 32° C. and then exposed over a solution saturated with ammonium chloride and potassium nitrate at 22° C. This gives a relative humidity of approximately 80 per cent. Ten days' exposure was necessary for equilibrium to be attained.

A further set of leaf segments after drying were leached with distilled water for three periods of 24 hours. By this treatment it was

expected that readily soluble salts in particular would be leached out. The leached segments were redried over calcium chloride at 32° C. and then exposed as described above. The water imbibed by the leached material is expressed as a percentage of the dry weight after leaching.

The response to treatment is again significant, the two treatments involving the supply of additional potash both giving an increase in the amount of imbibed water. The effect of leaching, however, is to reduce the amount of "imbibed water" to a common level for the three treatments. It appears, therefore, that the differences in the imbibed water of the unleached material are almost certainly due to differences in the amounts of readily soluble hygroscopic substances and are not directly concerned with the colloid substance of the cell.

(8) *Osmotic pressure of cell sap and leaf size.* It was thought that the factor responsible for an increase in cell size, and hence in leaf size, might be an increased osmotic pressure of the cell sap. To test this hypothesis determinations of the freezing-point of the expressed sap were made. Pairs of immature but rapidly expanding leaves were selected on eight plants on six dates. The youngest of each pair, generally the second leaf counting from the growing point, was cut for expression of the sap, and the other member of the pair labelled and the size which it ultimately attained determined when growth had ceased. Sampling was carried out at noon, and the leaves gathered frozen at once in liquid air and the sap expressed after thawing.

The differences due to treatment fail to reach the level of significance. Nevertheless, an attempt was made to correlate the freezing-point depression of the expressed sap with the ultimate leaf size. When allowance was made for the seasonal drifts in both leaf size and freezing-point of sap, a correlation coefficient of $+0.2168 \pm 0.388$ was obtained, which is not significant. Any relation between osmotic pressure of cell sap and ultimate leaf size will be a dynamic one, and the figures obtained do show differences in the right direction, an increased freezing-point depression accompanying the increase in ultimate leaf size.

Section 2

Transpiration experiments were conducted on the beet plants growing in pots. The amount of water transpired was determined by enclosing the pot in tinfoil and weighing at intervals. The amount of water transpired was added to the soil or sand of the pot daily. Two weighings were made each day, one at 10 a.m. and one at 4 p.m., and as the transpiration experiments were carried out early in the year

(January to March) we can consider 10 a.m.-4 p.m. as the day period and 4 p.m.-10 a.m. as the night period. Six plants of a series were always used in each experiment, mean values being extracted and the standard error of the means calculated. A porous cup atmometer was always run in parallel, and the water lost by the atmometer gives a useful measure of the evaporating power of the atmosphere during the course of the experiments. Most of the experiments were carried out in the greenhouse in which the plants were growing, but a few were done in the drier atmosphere of the laboratory. Stomatal frequency was determined for the same plants as were used for the transpiration experiments. In order to refer transpiration to a unit area basis, determinations of total leaf areas were made by tracing the outlines of the leaves on paper and determining the areas with a planimeter.

Table II contains a representative series of the results obtained with the "soil pot" plants, series G receiving no additional potash, series H receiving potassium sulphate and series I receiving potassium chloride. Transpiration for both day and night periods is given together with the weight of water in grams lost per hour by the atmometer. Relevant data for stomatal frequency are given also.

For each period series I shows a lower rate of transpiration than either series G and H, and the latter two series on no occasion differ significantly. The wide range of atmospheric conditions for which this holds is shown by the atmometer readings. The transpiration rate parallels almost exactly the stomatal frequency. Comparing "series" the conclusion to be drawn is that the differences in transpiration observed are occasioned by the differences in stomatal frequency. This is so for both day and night periods.

A similar series of results for the "sand pot" plants are incorporated in Table III. Series J received no additional potash, series K low potash, and series L high potash, the culture solutions applied to all three series being free from chloride.

Neither in transpiration rate nor in stomatal frequency do the three series of plants differ significantly from one another.

These plants grown in pots differ somewhat in their response to potash from those grown outside. The outdoor plants show both for the chloride and sulphate treatments a significant decrease in stomatal frequency. The plants in pots, grown in different soil, show a significant decrease only when potash is supplied as chloride, whilst the "sand plants", supplied with a culture devoid of chloride, are unaffected in this respect by the additional potash. Manifestly response to potash is affected by the supply of chloride. It seems likely that the

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TABLE II. Transpiration in g. per hour per square metre
of leaf with standard errors attached

Date 1934	Day period			Atmometer loss (g. per hr.)
	G (No potash)	H (K ₂ SO ₄)	I (KCl)	
26 Jan.	43.7 ± 1.72	44.7 ± 1.47	40.3 ± 1.88	0.342
27 Jan.	74.0 ± 2.80	74.7 ± 1.68	63.3 ± 1.98	0.550
28 Jan.	30.8 ± 2.38	34.3 ± 2.01	27.2 ± 1.08	0.208
24 Feb.	46.3 ± 2.19	44.9 ± 2.50	41.7 ± 1.11	0.417
27 Feb.	79.2 ± 4.27	75.7 ± 4.25	72.1 ± 2.81	0.733
Night period				
26-27 Jan.	15.4 ± 1.57	15.2 ± 1.51	11.7 ± 1.03	0.317
28-29 Jan.	10.7 ± 0.57	10.4 ± 0.77	7.9 ± 0.61	0.119
30-31 Jan.	29.3 ± 3.54	29.0 ± 1.91	18.3 ± 1.12	0.447
24-25 Feb.	15.6 ± 1.28	13.5 ± 1.32	12.3 ± 0.49	0.244
26-27 Feb.	17.4 ± 1.14	15.2 ± 1.57	13.0 ± 0.55	0.265
Stomatal frequency				
Series	No. of micro- scope fields counted		No. of stomata per sq. mm.	
	No. of leaves used		G	180.8 ± 7.9
G	12	138	H	181.4 ± 7.4
H	12	138	I	152.1 ± 6.6
I	12	139		

TABLE III. Transpiration in g. per hour per square metre
of leaf with standard errors attached

Date 1934	Day period			Atmometer loss (g. per hr.)
	J (No potash)	K (Low potash)	L (High potash)	
9 Feb.	54.5 ± 1.57	53.2 ± 4.50	55.0 ± 2.98	0.525
12 Feb.	54.0 ± 3.08	50.3 ± 5.04	57.2 ± 5.36	1.069
20 Mar.	32.9 ± 1.93	30.9 ± 2.44	27.7 ± 3.12	0.300
22 Mar.	25.8 ± 1.90	26.2 ± 1.79	26.5 ± 0.74	0.200
Night period				
9-10 Feb.	12.1 ± 0.72	11.7 ± 1.40	12.4 ± 1.33	0.228
11-12 Feb.	9.5 ± 0.57	9.4 ± 1.31	8.6 ± 0.93	0.131
20-21 Mar.	10.9 ± 0.63	11.0 ± 0.63	10.8 ± 1.24	0.217
21-22 Mar.	8.3 ± 0.53	7.7 ± 0.79	7.1 ± 0.81	0.128
Stomatal frequency				
Series	No. of micro- scope fields counted		No. of stomata per sq. mm.	
	No. of leaves used		J	167 ± 7.3
J	12	150	K	173 ± 8.5
K	12	144	L	157 ± 5.7
L	12	152		

garden soil of the outdoor plots is well supplied with chloride, so that here no different response to the chloride and sulphate treatments is obtained.

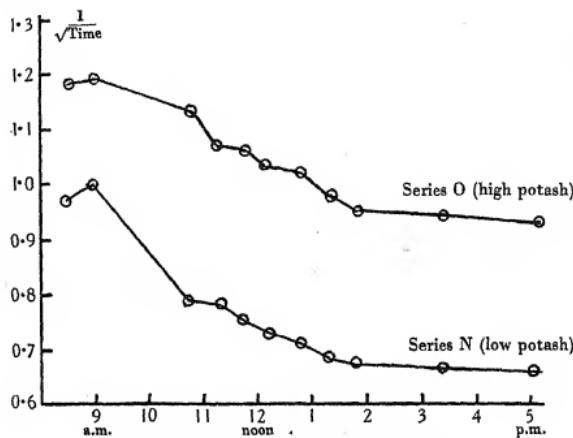


Fig. 1. Buckwheat, 1 June 1934.

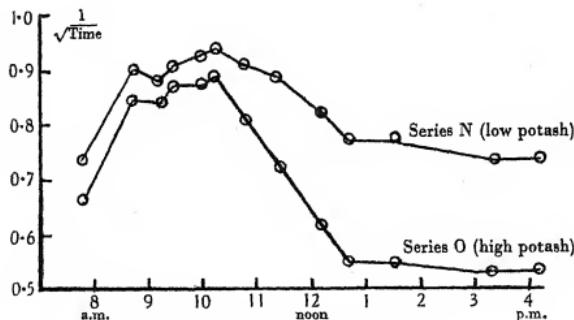


Fig. 2. Buckwheat, 5 June 1934.

Diurnal changes in stomatal aperture. Changes in the aperture of the stomata were investigated by using a porometer fixed to the underside of the leaf and operated by means of a constant level siphon drawing air through the leaf. Always an experiment was set up in the evening, and readings taken the next day. Beetroot plants in pots and buckwheat in water cultures were used. In all the experiments the leaves used were still joined to the parent plant. The reciprocals

of roots of intervals between bubbles were taken as being proportional to stomatal aperture (Darwin, 1916; Knight, 1917).

Buckwheat. The comparison here was made between the plants in water culture receiving 132 parts of potash (K_2O) per million parts of

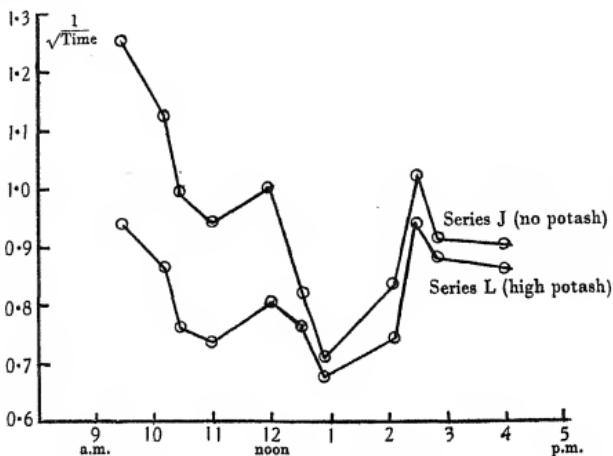


Fig. 3. Seakale beet, 28 March 1934.

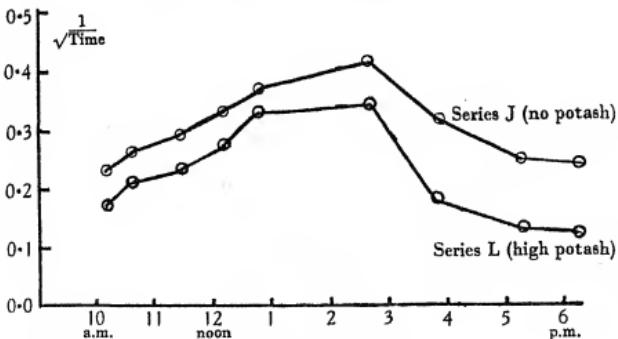


Fig. 4. Seakale beet, 13 March 1934.

culture (series N) and those receiving 658 parts of potash per million parts of solution (series O). The first leaf proper of the plant was used in the tests, so that the comparison was made between leaves of identical age. The graphs in Figs. 1 and 2 illustrate the diurnal changes in stomatal aperture observed. In both cases maximum stomatal opening is exhibited early in the day, at approximately

9.30 a.m. in one case and at 8.30 a.m. in the other. These experiments were conducted in a window with a northern aspect so that they received some early morning sun, and this probably accounts for the early hour at which the maximum stomatal aperture was observed. The curves for the plants of the two series are of a very similar type, changes in one being accurately reflected on changes in the other. In Fig. 1 the part of the curve corresponding with stomatal closure is somewhat steeper for the plant of series N, but in Fig. 2 a reversal of this takes place. The level of the curve has no significance and is probably due mainly to slight differences in the sizes of the porometers.

Beet. The comparison here was made between plants in sand culture receiving no potash (series J) and those receiving 658 parts of potash (K_2O) per million parts of culture solution. The progress of stomatal opening and closing is illustrated in Figs. 3 and 4. Here again the curves for the two series parallel each other very closely. Fig. 3 is a record of the change in stomatal aperture of plants which had been exposed to early morning sun. Indications of a steeper curve for series J on Fig. 3 are not supported by the results on which Fig. 4 is based.

Manifestly neither with the beet nor with the buckwheat was the additional supply of potash exerted to any appreciable effect upon stomatal opening and closing.

DISCUSSION

The data from the plants of the outdoor plots must form the main basis of the discussion. The additional potash has here resulted in increased growth. This is reflected in the increased number of leaves on each plant and in the production of larger leaves. It seems clear that the increase in leaf size has been effected through an increase in the size of the individual cells, and the indications are that the number of cells constituting the leaf is unaltered. The manner in which the cell size is influenced is not so clear. Cell expansion is not a simple process but is itself capable of further analysis. For each species upper and lower limits of cell size will be determined by genetical constitution. Between these limits each species will exhibit a degree of plasticity. For cell expansion to occur a certain degree of turgor is necessary, maintaining the cell walls in a condition of tension. This turgor is developed and maintained through the production of osmotically active substances. Turgor alone is not sufficient to bring about cell expansion. The cell walls must possess the power of extension.

In this experiment it was not possible to demonstrate a significant correlation between osmotic pressure of the expressed cell sap and the ultimate size of the leaves. This is not to say that such a correlation does not exist, but the correlation coefficient obtained can only be described as suggestive.

The increase in the size of the cells is considered to be the primary effect of the additional potash. An increase in the size of individual cells has a far-reaching effect upon the properties of the leaf. It is likely that with an increase in cell size the proportion of the cell occupied by the vacuole increases, resulting in an increased water content. In the experiments described here a real increase in water content of the leaves was found when the potash supply was increased, the increase being due, it is suggested, to the increased cell size.

No indication has been obtained that the additional potash has directly increased the ability of the leaves to withstand water loss. The determination of "imbibed water" gives no indication of an increase in the water-holding properties of the leaf colloids, the increase in imbibed water which was observed being due entirely to readily soluble substances, probably mainly, soluble hygroscopic salts. The increase in cell size, resulting in a proportionate decrease in stomatal frequency, has an important effect upon the water relations of the leaf. With the pot plants a reduced stomatal frequency was obtained only when the additional potash was supplied as chloride. In all the transpiration experiments, comparing different series of plants, transpiration was proportional to stomatal frequency. If these results are applied to the outdoor plants we can infer that the additional potash has effected a reduction in transpiration, due to its effect upon stomatal frequency. The output of water per leaf will be unaltered, whilst the amount transpired per unit area of leaf is reduced. A decrease in stomatal frequency will affect all phases of the physiology of the leaf in which gaseous exchange through the stomata plays a part. This may apply to assimilation, especially when carbon dioxide is the "limiting factor".

Although the responses to treatment which have been described are undoubtedly responses to the additional potash supply, the actual response is not proportional to the potash content of the leaves. In the plants in pots a response was obtained only when the potash was supplied as chloride. The nature of the response to the potash, therefore, seem to be influenced by the chloride supply, but the way in which the chloride exerts an effect is not clear. It is noticeable,

however, with the outdoor plants that, whilst the chloride plants have given as great a response as the sulphate plants, the potash content of the latter is much greater than the potash content of the material from the chloride treatment. With these plants too the response to potash appears to be affected in some way by the chloride ion.

As far as the outdoor plants are concerned, there is a suggestion of physiological dryness in the no-potash plants. Apart from the actual water content, which is lower in these plants, the smaller cells and greater stomatal frequency are characters frequently associated with a water deficiency. Under conditions of water shortage the cells soon cease to expand (Zalenski, 1928). The production of the smaller cells in the no-potash plants is the only indication of a physiological dryness in the potash-deficient plants, such as Wallace has suggested for fruit trees.

SUMMARY

1. Experiments set up to elucidate the connexion between potash supply and the water relations of the leaves of seakale beet are described.

2. The additional potash produces an increase in the water content of the leaves when expressed on an area basis, but the increases do not always reach the level of significance. When expressed on a dry-weight basis, variations in the amount of dry matter in one case mask a real increase in water content.

3. The content of potash in the leaves is increased, the increase being greater when the extra potash is supplied as sulphate than when it is supplied as chloride.

4. The contents of sulphate and chloride on the leaves are increased only when the potash is supplied as sulphate or chloride respectively.

5. The increase in the potash supply effects an increase in the amount of water imbibed by dried-leaf material. The increase appears to be due solely to the presence of an increased amount of readily soluble hygroscopic substances, rather than to any alteration in the properties or amount of the colloidal substances of the leaf.

6. The additional potash supply has effected an increase in cell size and leaf size and a decrease in stomatal frequency.

7. With plants grown in pots differences in transpiration rates between series are accounted for entirely by differences in stomatal frequency. There is no evidence that the potash affects transpiration except through its effect upon stomatal frequency.

8. Diurnal changes in stomatal aperture are unaffected by the level of the potash supply.

9. It was not possible to show that the osmotic pressure of the cell sap in the immature leaves was the factor determining the ultimate size attained by the leaves.

ACKNOWLEDGEMENTS

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AN ADAPTATION OF HALDANE'S GAS-ANALYSIS APPARATUS

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(With 1 figure in the text)

FOR studying the respiratory gaseous exchange in plants Haldane's gas-analysis apparatus (Haldane, 1912) is usually employed. No doubt, due to the ingenious construction of the KOH bulb which serves also as a manometer, the instrument is at once accurate and convenient to manipulate.

In the course of investigations on the gas storage of tropical fruits, the original Haldane apparatus has been slightly modified so as to render it convenient for R.O. measurements in plants. For the simultaneous determination of the oxygen absorbed and the CO₂ liberated, the plant material is usually enclosed in respiration chambers which provide for the removal of small amounts of gas for analysis. When only R.O. is needed, analysis of a gaseous sample suffices to give the desired ratio, but in case it is required to know the absolute amounts of oxygen absorbed and CO₂ evolved, it is necessary to determine the total volume of the gas mixture in the respiration chamber as well as the percentages of the single constituents. Since a direct calibration of the respiration chamber after the introduction of the plant material is in most cases impracticable, recourse has to be taken to some indirect method. By introducing slight changes in the construction of the Haldane apparatus, the instrument has been rendered suitable for calibrating the respiration chambers as well.

In the construction of the apparatus (Fig. 1) the potassium pyrogallate bulb has been replaced by a phosphorus bulb (*D*) of the type employed by Carpenter (1915). This bulb is similar to the "combustion" pipette used by Haldane (1912), except that the ignition tubes inside the pipette have been removed and a water-levelling bulb added (Singh & Mathur, 1935, 1936). An alkaline solution of pyrogallol, when used as an absorbent for oxygen, requires to be frequently renewed, thus involving much time and expense. It is preferable to use thin moist rods of phosphorus which allow many hundreds of analyses to be carried out in succession. The

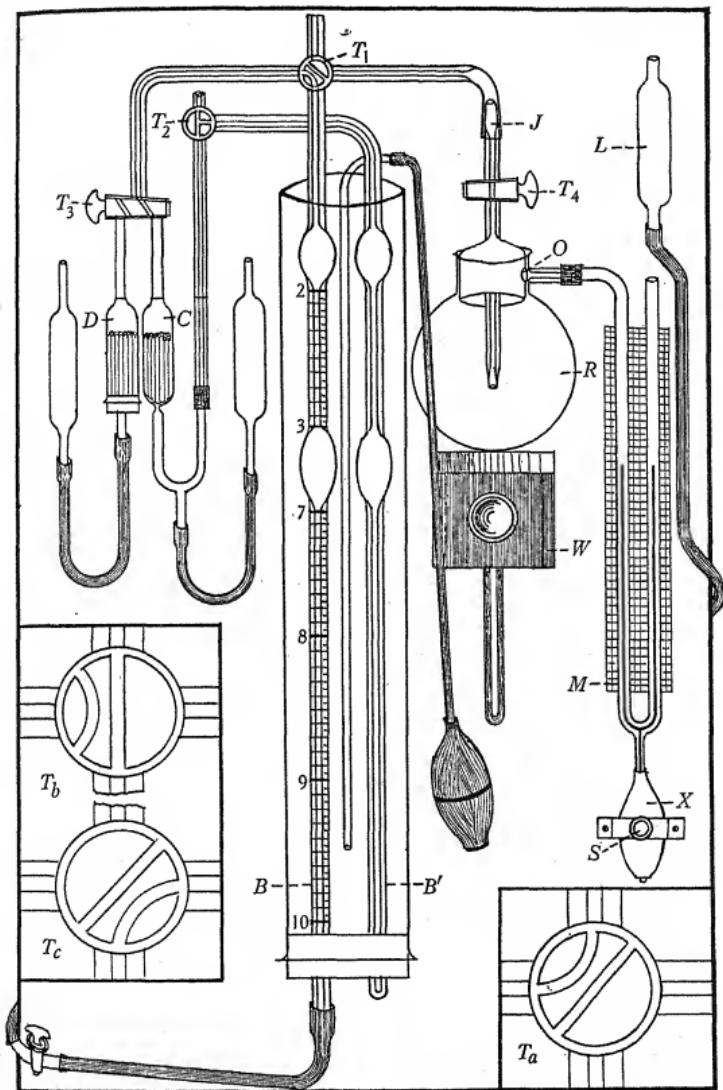


Fig. 1. An adaptation of Haldane's gas-analysis apparatus.

KOH bulb (*C*) is of the usual Haldane type. The measuring burette (*B*) possesses two bulbs which alternate with portions graduated in hundredths of a ml. A compensation burette (*B'*) is also provided. The respiration chamber (*R*) carries a ground-glass stopper with a tap (*T*₄). A manometer (*M*) is connected to the side tube attached to the respiration chamber. A strip of squared paper is mounted behind the manometer to record the differences of pressure in the respiration chamber. The manometer contains paraffin, the liquid adjustments being made by means of the screw (*S*), which presses the rubber reservoir (*X*). The respiration chamber rests on a wooden bracket (*W*) which can be easily moved up or down by means of a screw arrangement. The whole outfit is mounted on a heavy wooden stand for convenience in manipulation.

The manipulation is as follows. After the introduction of the plant material and a suitable gas mixture, the respiration chamber is worked on to the ground joint (*J*) in the apparatus (Fig. 1) and connected with the manometer (*M*). Now the ground-glass stopper of the respiration chamber is turned so that it communicates with the manometer (*M*) through the orifice (*O*). Keeping the tap (*T*₄) closed, the tap (*T*₁) is turned in the position (*T*_a) and the taps (*T*₂) and (*T*₃) in the positions shown in Fig. 1, and the potash levels in the KOH bulb are set, after which the tap (*T*₁) is turned in the position (*T*_b). Now the tap (*T*₄) is opened, and the liquid in the manometer adjusted to the same level in both the limbs. By raising the levelling bulb (*L*), the mercury in the burette (*B*) is brought up to the tap (*T*₁), which is subsequently turned in the position (*T*_a). The bulb (*L*) is lowered and 2-3 ml. of gas from the respiration chamber withdrawn into the burette (*B*). Subsequent to this, the tap (*T*₁) is turned in the position shown in Fig. 1 and the lowering of pressure in the respiration chamber following the withdrawal of the gaseous sample noted. The volume of the gas withdrawn from the respiration chamber into the burette (*B*) is also noted, the potash levels in (*C*) being kept set. From these data the volume of gas, say *x*, is easily computed:

$$x = \frac{VH}{h},$$

where *V* = volume of the sample withdrawn at the atmospheric pressure;

H = atmospheric pressure in mm. paraffin;

and *h* = decrease in pressure in the respiration chamber in mm. paraffin.

For analysing a sample for CO₂ and oxygen the procedure, in brief, is as follows. At regular intervals the respiration chamber is removed from the water bath and placed in the position shown in Fig. 1. No connexion is made with the manometer and the orifice (O) is kept away from the side tube. The air in the apparatus is first freed from CO₂ and oxygen in order that all the capillaries may be filled with nitrogen. After the potash levels have been set, a sample of respired air is withdrawn from the chamber (R) into the burette (B), two to three samples being usually rejected with a view to washing the connexions with the expired air. Further details of procedure have been described in detail by Haldane (1912) and Carpenter (1915).

Attention has recently been drawn by Blackman & Parija (1928) and Gustafson (1929) to the existence of several metabolic groups among fruits of the same chronological age. As shown by Gustafson (1929), during the ripening of tomatoes the colour usually offers a general indication of the physiological age of the fruit. In Table I are

TABLE I. *Carbon dioxide/oxygen ratios during ripening*

Colour	ml. (N.T.P.) per kg. per hour		
	Carbon dioxide	Oxygen	R.Q.
Green	17·7	17·5	1·01
	19·6	17·3	1·13
Yellow-green	22·3	21·3	1·05
	24·1	23·7	1·02
Green-orange	30·1	29·8	1·01
	27·8	27·6	1·01
Orange-red	21·0	19·8	1·06
	11·9	9·9	1·20
Red	8·8	7·1	1·24
	7·4	6·1	1·21

presented some respiration data obtained for a single tomato fruit over a 10 day period. The fruit was picked from the orchard when it had attained more or less the maximum size, and was placed in the respiration chamber maintained in a water bath at a temperature of $27 \pm 0.2^\circ\text{C}$. The respiration chamber was connected with an aspirator and a slow current of CO₂-free air drawn over the fruit. At 24 hour intervals, the respiration chamber was disconnected from the aspirator and was kept closed for 5–6 hours in each case, after which the analyses of the gas mixture were carried out. During senescence, the ratio CO₂/O₂ started with a value slightly higher than unity and gradually rose to 1·24, when the fruit had attained a red colour and was fully ripe.

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ON CERTAIN UNIQUE FEATURES OF
THE GYNOECIUM IN NOLANACEAE

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(With seven figures in the text)

OF the three genera recognized to-day as composing the small family Nolanaceae, viz. *Nolana*, *Alona* and *Dolia*, the first to become known was *Nolana*, and of the forms included in this genus *N. prostrata* L. and *N. paradoxa* Lindl. were the first to be introduced into this country.

Lindley, in his account of *paradoxa* (1824), describes the pistil as consisting of twenty separate ovaries or nucules of which about fifteen usually abort. Those that mature, when ripe, become detached separately from the part on which they are borne. Each is described as being one-celled and one-seeded and as showing on the face of the large attachment scar a single central small scar or areola.¹ The pistil of the earlier-known *prostrata*, on the other hand, has only five original nucules set regularly round the single "gynobasic" style filament, and these Lindley describes as being four-celled and four-seeded and as having four areolae on the attachment scar. These features led Lindley to conclude that the pistil in *prostrata* as in *paradoxa* is made up of twenty ovaries, but that, whereas they are all separate in *paradoxa*, they are conjoined in fours in *prostrata*, each group of four constituting one nucule. Lindley further suggested that *paradoxa* stood in the same relation to *prostrata* as *Malope* to other Malvaceae, and he concluded that the characteristic features of the fruit were paralleled in the Boraginaceae.

It follows from the above statements that Lindley employed the term nucule merely as a general descriptive term for the separate portions of the ripe fruit, not as signifying a particular carpellary construction. In this connexion it should be borne in mind that the term carpel was coined first by Dunal (1817). It is therefore more than likely that at the time Lindley wrote the above account the term had not acquired its present precise significance and was quite possibly

¹ Taken by Lindley to be the point of attachment of the style but indicating, in fact, the position of the broken funicle.

not in general use.¹ At any rate Lindley does not use it here. There is no reason to doubt, however, that by the term ovary he did intend here to indicate structures morphologically equivalent and that he regarded this structure in both these *Nolana* forms as consisting of a single carpel. Indeed, this is put beyond question by his comparison of the gynoecium of *paradoxa* with that of *Malope*, for he describes "the carpella" of the latter genus as aggregated and distinct (1830, p. 33).

Now Lindley's conception that the five nucules of the *prostrata* gynoecium are made up of twenty ovaries, i.e. carpels, appears to have been based on the presence (1) of as many as four areolae on the attachment scar on each nucule, and (2) of parenchymatous partitions between the ovules so that each of the four in one nucule lies in a separate chamber.

Now although we can to-day point to a striking case, viz. *Eschscholtzia* (Saunders, 1925, pp. 133-5; 1927, pp. 616, 617), in which particular groups of carpels remain adherent and so form a distinct structural unit, it scarcely needs to be emphasized that outward appearance without other evidence cannot be taken as establishing beyond doubt this extremely rare type of construction.² It therefore becomes necessary to investigate further the nature of the *Nolana* nucule and to set out in more precise terms the mode of construction of the gynoecium in the above types. Since, however, the gynoecium of the later-discovered, but to-day more familiar, *atriplicifolia* is similar to that of Lindley's *paradoxa* the former type will be treated in place of *paradoxa* in the following account.

The development of the flower in the two species *prostrata* and *atriplicifolia* follows the same course in so far (1) that the midrib bundles for sepals, petals, and the one whorl of stamens turn out from the central cylinder independently and in strict alternation; (2) that the sepals are furnished with commissural lateral veins (formed from the same delimited portion of the central cylinder as the petal midribs though the latter bundles turn outwards later than the conjoined laterals) and also with true lateral branches derived from the midrib; and (3) that the base of the gynoecium is surrounded by a ring of non-vascular disc tissue. From this point, however, it will be necessary to consider the two species separately.

¹ This supposition receives support from the following comment on the flower of the Pomegranate written by Lindley six years later. "Now that the structure of this part is well understood we know that an ovary consists of one or several pericarpial leaves called carpella . . ." (1830, p. xxx) (the italics are mine).

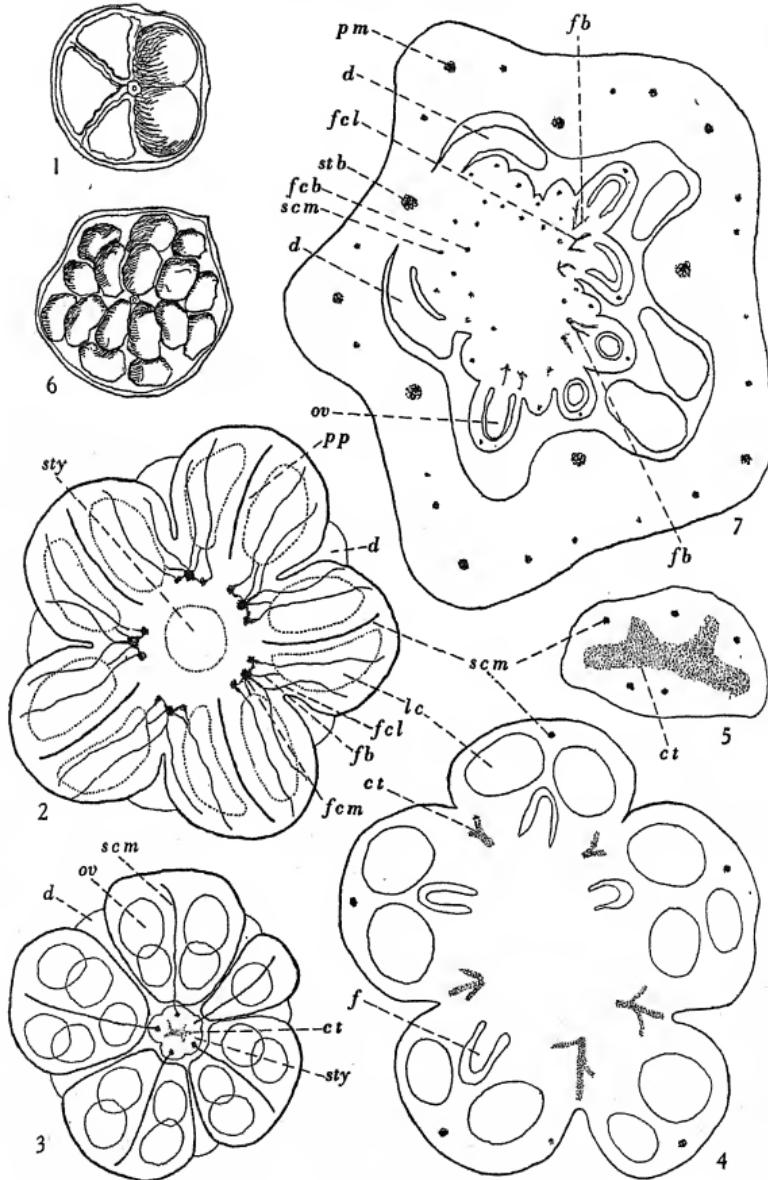
² Also characteristic of some syngnathous types, e.g. Umbelliferae.

N. prostrata. Nucules 5, antepetalous, several-seeded with a corresponding number of areolae (Figs. 1-5).

After the emergence of the bundles for the sepal, petal and staminal whorls, as described above, the residual vascular tissue becomes consolidated into a single bundle on each petal radius which turns outwards in the midline of the corresponding nucule, and a single bundle on each alternate radius which remains central. The former bundles constitute the midrib bundles of five sterile solid carpels. They pass up in the outer wall of the nucule, generally remain unbranched, and turning inwards over the closed loculi enter the style filament which is at first terminal and only becomes lateral as development proceeds. The residual bundles on the alternate radii give rise to considerable branch systems. From each of these bundles two or three strands turn outwards both to right and to left. These branches run up in the adjacent half of the outer wall of the nucule on either side. Other branches, also given off to right and to left, supply the tier of ovules in the adjacent half of the same two nucles. This whole system is interpreted as that of a fertile semi-solid carpel. The gynoecium is therefore held to be composed of two whorls of five carpels and each nucule to be formed of $\frac{1}{2}$ carpels. Typically each nucule contains six ovules arranged in two tiers of three, the vascular supply of one tier being derived, as explained above, from part of the system of the semi-solid carpel to the right, that of the other tier similarly from the corresponding part of the system of the carpel on the left.

Though comparable in composition and mode of origin¹ with the coccus of most Malvaceae and with the individual fruits of other pseudo-apocarpous types (e.g. Crassulaceae and some Rutaceae), the nucule differs from all such forms in that the loculus is partitioned by parenchymatous tissue in such a way that each ovule lies in a separate chamber. In this exceptional feature *N. prostrata* stands in somewhat the same relation to the ordinary type with unchambered loculi as the crucifer *Bunias Erucago* to the bulk of that family, although the developmental process is somewhat different in the two species. In *B. Erucago* the two normal loculi become converted into four chambers through the zigzag direction taken by the sheet of tissue formed of the two fertile carpels (i.e. the replum) which comes into contact, and fuses, now with one lateral wall of the ovary (i.e. one sterile carpel) and now with the other. In this way it separates the four ovules from one another. That is to say, the chambering is due to

¹ Through radial splitting from without inwards of the fertile carpels.



Figs. 1-7.

the peculiar conformation of the fertile carpels. In *N. prostrata*, on the other hand, it is the sterile carpels which form the partitioning tissue. These carpels consist of a radial sheet of tissue in which the midrib runs outwards. This radial sheet extends from the central parenchyma to the outer wall of the ovary and remains continuous with these tissues throughout. By this means the primary loculus is divided vertically in half. This partitioning parenchyma is also continuous laterally between the superposed ovules with the tissue of the fertile carpel on each side, hence the ovules develop in separate chambers.

Explanation to Figs. 1-7.

Figs. 1-5. *Nolana prostrata* L. Fig. 1. A fruit showing two nucules on the right and on the left the large scars left after the removal of the other three. (The areolae on the scars are not indicated.) In the centre the scar of the style column. Figs. 2, 3. The gynoecium halved transversely and rendered transparent. Fig. 2. The lower half viewed from below showing the median partition halving the loculi, the unbranched midribs of the sterile carpels and, on the alternate radii, the much branched vascular system of the fertile carpels. Fig. 3. The upper half viewed from above. The nucules are now disjoined from one another through complete radial splitting of the sterile carpels, but are held in position by the underlying disc tissue. In each nucule the uppermost ovules are seen through the wall of the ovary. In the centre the "gynobasic" style filament. Within the filament the bundles of the five sterile carpels and a core of conducting tissue. Fig. 4. Transverse section from the middle region of the gynoecium showing the older, lower chambers of the loculi from which the ovules have dropped out and, three of the upper, younger chambers with developing ovules. On the alternate radii the branching rays of conducting tissue which extend almost to the exterior. Towards the outside in the mid-line of the nucules the sterile carpel midribs. (The vascular system of the fertile carpels which at this level shows no regular pattern is not represented.) Fig. 5. The style filament. Towards the periphery the five sterile carpel midribs. In the centre the core of conducting tissue. Figs. 6, 7. *N. atriplicifolia* hort. Fig. 6. A fruit of sixteen nucules. In the centre the scar of the style filament. Fig. 7. A slightly oblique section of a young bud after removal of the calyx. On the outside the corolla-androecium tube. Within the tube on the right portions of three anthers, and on the left portions of the disc not yet disjoined from the corolla tube and gynoecium. In the centre the gynoecium showing various stages in the development of the nucules (ovaries) of which the first to expand are those in line with the petals. On the left the vascular system as it appears below the level of enlargement of the ovaries. The outer ring of bundles become the sterile carpel midribs. Those of the inner ring on alternate radii furnish the fertile carpel systems. On the right where the ovaries have enlarged the sterile carpel midribs are seen in the outer wall. The fertile carpel bundles are in process of giving rise to the branches which enter the lateral walls of the ovaries and to those which supply the ovules. *ct*, conducting tissue; *d*, disc; *f*, funicle; *fb*, funicle bundle; *fcb*, fertile carpel bundle; *fcl*, fertile carpel laterals; *fcm*, fertile carpel midrib; *lc*, lower chamber; *ov*, ovule; *pm*, petal midrib; *pp*, parenchymatous partition; *scm*, sterile carpel midrib; *stb*, stamen bundle; *sty*, style filament.

Only the sterile carpel midribs are prolonged into the smooth cylindrical style filament. In this respect it offers a contrast with that of the Malopeae. For in this malvaceous group it is the conjoined systems of the two fertile half carpels which are from the first directly prolonged into the filament as a single bundle. Only at a much later stage does the sterile carpel midrib extend over the top of the loculus far enough to join up with the united systems of the two fertile half carpels (Saunders, 1936). The filament differs also from that of the Malopeae in being solid, a central five-angled core of conducting tissue wholly filling the central canal. This tissue extends downwards below the level of origin of the column forming five rays which extend outwards in the midline of each fertile carpel. These carpels, thus divided from within outwards as well as from without inwards (see above), become split almost completely in two, the halves being connected at this level by only a narrow band of two or three cell layers. Each of these main conducting tracts connects by lateral tracts with the ovule chambers on each side.

The terminal discoid stigma may be depressed and five- (or four-¹) furrowed or convex and unfurrowed according as the suturing of the carpel tips is plainly defined or is quite even and invisible.

As the fruit ripens the nucules become detached separately. This comes about through the tearing of the tissues in such a way as to leave behind attached to the axis the style filament (which may persist and wither *in situ*) and the ventral lower portion, together with the placental vascular system, of the fertile carpels. Since the seeds in the individual nucule are separated by bands of parenchyma and are developed two abreast in tiers of three the scar, after detachment, on both axis and nucule is exceptionally large. Within this scar there may be seen as many as six smaller scars (areolae) marking the broken funicles.

From the above it will be apparent that the several-seeded, several-chambered *prostrata* nucule is a unique structural unit, differing fundamentally in construction from the one-seeded nutlets of the Boraginaceae and Labiateae, the nucule being composed of $\frac{1}{2} \frac{1}{2}$ carpels, the nutlet of $\frac{1}{2} \frac{1}{2}$ carpels.

N. atriplicifolia. Nucules (typically) 25, one-chambered and one-seeded with one areola (Figs. 6, 7).

The arrangement of the numerous nucules, as remarked by Lindley, resembles that seen in the malvaceous genus *Malope*. In

¹ If, as is not uncommon, one of the five sterile carpels is weaker than the others and scarcely contributes to the stigmatic surface.

both types the ovaries arise in five antepetalous groups, those of each group developing successively and coming to overlie one another as each younger one enlarges at a higher level than its older neighbours. The succession is less regular, however, in *N. atriplicifolia*, and this fact coupled with the distortion which follows renders analysis of the ground plan even more difficult than in similar malvaceous types. If, however, sections are taken at a sufficiently early stage, it at once becomes clear that the vascular scheme is similar in the main to that of the Malopeae. Midrib bundles for the numerous sterile carpels leave the central cylinder in succession, those in line with the petals, as in *Malope*, being the first to turn outwards. After the emergence of these bundles the remaining vascular elements become arranged in a ring of bundles on the intervening radii. Each of these bundles gives rise to branches to right and left and so comes to an end. These separate systems supply a corresponding number of fertile carpels. Owing to the fact that the enlargement of the ovaries (due to the formation of the loculus and the development of the ovule) takes place at varying levels, the branches given off on one side of each fertile carpel bundle arise at a different level from the corresponding ones on the other side. Of the branches formed on the one side the first runs outwards in the lateral wall of the adjacent ovary on that side. Later another extends laterally until it meets, and coalesces with, the corresponding branch from the adjacent fertile carpel bundle on that side. The two form a single placental strand, which supplies the solitary ovule and thus comes to stand on the same radius as the sterile midrib belonging to the corresponding ovary. Each individual ovary is thus constructed of a sterile carpel conjoined with half the fertile carpel on each side. That is to say, the *atriplicifolia* nucule and the *prostrata* nucule, despite the disparity in size, are morphologically equivalent. Both are composed of $\frac{1}{2}$ carpels. That this is so is further confirmed by the fact that now and again an *atriplicifolia* nucule may occur which is two-seeded. Such nucules are larger than the rest, have two areolae, and exhibit the same longitudinal chambering as those of *prostrata*. Also, as in *prostrata*, but in this respect differing from the Malopeae (see above), only the sterile carpel midribs are prolonged into the "gynobasic" style filament which differs from that of *prostrata* in being longitudinally ridged. The filament terminates in a sinuous crest-like stigma giving an appearance of five lappets. The conducting tissue, as in *prostrata*, forms a five-angled core and rays out below the style filament in the midline of the fertile carpels, so that the nucules

become completely split in half except for some two or three layers of cells which finally rupture as the nucules ripen.

The loosening of the individual nucules is brought about in the same way as in *prostrata* through the completion of the median radial splitting of the fertile carpels and the rupture of the tissues on the ventral face, so that the funicles break away from the placental bundles which are left behind on the axis together with the style column.

Brief reference may be made to the form of the gynoecium in individuals of crossbred origin. Flowers of *prostrata*-*atriplicifolia* crossbreds often produce both types of nucules, a few which are small and one-seeded being irregularly sandwiched between some five or more of the large *prostrata* form. In the progeny of crossbreds nucules of monstrous shape are common owing to failure to complete the median radial splitting of individual fertile members, with the result that two neighbouring nucules remain connected by a narrow neck.

It remains to summarize the conclusions which follow from the preceding account.

SUMMARY

1. The similarity in appearance of the gynoecium in *N. atriplicifolia* and in the section Malopeae of the Malvaceae is due to a similar construction, the partial fruits (nucules of *Nolana* and cocci of the Malopeae) being composed in both instances of $\frac{1}{2}\frac{1}{2}$ carpels.

2. *N. prostrata* stands in similar relation to *N. atriplicifolia* in regard to the gynoecium as pentamerous members of the Hibisceae to the Malopeae among Malvaceae.

3. The partial fruit (nucule) of *N. prostrata* shows partitioning of the loculus in such a way that each ovule lies in a separate chamber as in the crucifer *B. Eruca*. These two types differ, however, in that the partitioning is caused by the tissues of the fertile carpels in the crucifer and by that of the sterile carpels in *N. prostrata*.

4. The ripe nucules of *Nolana* become detached through complete longitudinal median splitting of the fertile carpels and rupture of the funicles and tissues of the ventral face of the ovary below the style filament in such a manner that the placental bundles and style filament are left behind on the axis.

5. The equivalence of the several-seeded nucules of *N. prostrata* and the one-seeded nucules of *N. atriplicifolia* is further established by the fact (a) that nucules of *atriplicifolia* are occasionally two-seeded

and partitioned, and (b) that one-seeded and several-seeded nucules occur together in crossbreds between the two species.

6. Monstrous double nucules which arise through incomplete radial fission of the fertile carpels are also met with in plants of cross-bred origin.

The drawings of Figs. 1 and 6 were made by Miss D. F. M. Pertz to whom I am much indebted.

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MICELLAR STRUCTURE OF THE TRACHEIDE
WALL IN CERTAIN WOODS, IN RELATION TO
MORPHOGENETIC AND MECHANICAL
FACTORS

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(With Plates VII and VIII and 6 figures in the text)

INTRODUCTION

THE sporadic occurrence of minute, roughly parallel striae and cracks in woody cell walls is a familiar fact, and their association with micellar or molecular structure has been suggested by previous investigators (Haberlandt, 1914; Robinson, 1921). That the relatively constant slope of such striae and slits is an indication of organized ultra-microscopic, crystal-like structure was, in fact, put forward by Nägeli (1862, 1864, 1879) and by Nägeli & Schwendener (1877), who even observed that the directions of greatest swelling and optical elasticity were connected with the same feature.

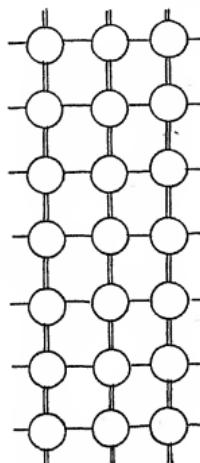
Jaccard & Frey (1928) have recently reinvestigated the slope of the fibrillae of a few hard-wood and soft-wood species, in relation to longitudinal swelling and shrinkage, in compression and tension, spring and summer woods. But Hartig seems first to have noted an abnormal longitudinal contractivity of compression wood.

According to Jaccard & Frey the mean slope of the fibrils is typically less steep in compression than in tension wood. The same investigators also found that summer and tension wood, and spring and compression wood, respectively, showed like characters: in the former the elements were longer, with steeper micellar spirals, than in the latter. It was concluded that the differences of fibrillar slope in Rothholz and Zügholz were due to growth-rate rather than to direct mechanical influences.

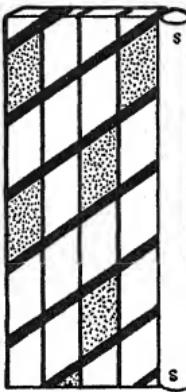
The work of Welch (1926) on Swamp Kauri, and of Kelaney & Searle (1930) on the chemical sectioning of plant fibres, should be referred to in connexion with the "seasoning slits" of the cell wall discussed in this paper. Moreover, recent X-ray analyses (1920, 1928) of the plant cell wall have thrown much additional light on the problems of molecular and micellar wall structure. But whether the constituent micellae be regarded as "crystalline" units or "gel"

substances matters little, since the colloidal units of the gel may themselves consist of orientated molecules—i.e. crystals.

The idea of parallel (spiral) molecular, or micellar, strings, firmly bound together by "primary valencies" in a longitudinal direction, with certain "residual valencies" in a roughly transverse direction will, however, be found helpful to a clear understanding of the relation between fibrillar structure and the phenomena of swelling



Text-fig. 1.



Text-fig. 2.

Text-fig. 1. Diagram of molecular patterning (vertical section) in plant cell wall, after Sponsler. Primary valencies denoted by ||, residual valencies by —.

Text-fig. 2. Diagram of hypothetical micellar structure, after Forsaith. Cellulose units shown white, lignin shaded, adsorbed water black. SS = silica rod, as indicated by Brown.

and shrinkage of a wood (see Text-figs. 1, 2). And it was upon such a picture of cell-wall structure that the present investigation was based.

Assuming that the longitudinal molecular or micellar series are firmly bound together by electrochemical affinities, it is evident that adsorbed water will tend to penetrate between the spiral lines of the former, rather than in transverse planes (see Text-fig. 2). Hence, if the longitudinal fibrils of a cell wall happen to run quite parallel to the long axis of the cell, there should be little or no expansion or contraction in a *longitudinal* direction with changes of moisture content. On the other hand, if there happens to exist an obliquely spiral arrangement of the fibrillar series, the adsorption of water molecules between

them should create a longitudinal component of dimensional change more or less proportionate to the relative inclination of the fibrils to the vertical wall. Haberlandt (1914, p. 163) quotes Sonnataag as having suggested the same thing, so far, at least, as the adsorption of water is concerned, and elsewhere (p. 550) mentions the effect upon expansion and shrinkage.

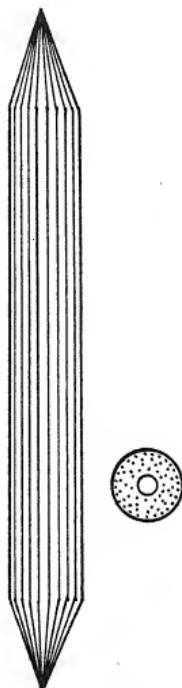
Hence, assuming that the inclination of the fibrillar or micellar series is truly indicated by the microscopic cracks that are sometimes met with in coniferous woods, it follows that either percentage shrinkage or expansion, in both longitudinal and transverse directions, should show a clear correlation with the relative inclination of such cracks; for convenience, hereafter termed angle θ° . To determine this relation—if existent—for a number of different species was the main object of the present enquiry; though, secondarily, growth features were also taken into consideration.

On the other hand, the longitudinal siliceous strands in the cell wall, noted and described by F. Brown (1920), might be expected to exert a binding effect over dimensional changes in the longitudinal direction (see Text-fig. 3).

Most relevant of all to the present investigation, however, is the recent important contribution to the theory of micellar structure by R. D. Preston (1934), on the *Pinus* tracheide under X-ray analysis. The paper in question was, unfortunately, published after the completion of the present investigation. But it will be seen from the following summary of co-relative findings, that the two papers are more or less complementary and corroborative, in so far as they cover the same ground. Thus Preston concluded that:

1. The inclination of the micellae to the long axis of the cell was rather less on tangential than on radial walls.
2. The micellae formed a spiral at a fairly constant angle of inclination (which he also terms θ) to the long axis, sometimes left-, sometimes right-handed.

Text-fig. 3. Diagrammatic representation of the siliceous skeleton of a fibre, after Brown.



3. The mean inclination of the micellae in tangential walls of the spring-wood tracheides of any ring was approximately the same.

4. The average inclination of the micellae on the corresponding radial walls varied from one annual ring to the next, thus:

$$L = K \cot \theta,$$

$$L = KB \cot \theta,$$

where L = average length of tracheides of spring wood of the ring,

B = average radial width, and

K = a constant.

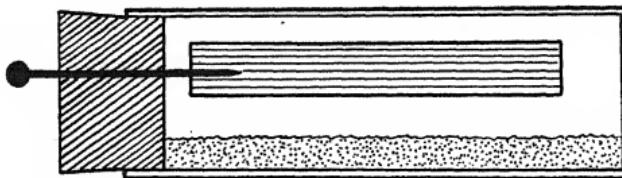
5. The cambial initials were analogous to spirals, increasing in length at constant girth.

6. The micellae in a new layer deposited on an old wall were so orientated by the *old wall* that they lay parallel to the micellae in the latter.

7. Photos showed clearly that cracks in the wall parallel to the major extinction positions were perpendicular to the rows of spots on the X-ray pictures, and that the angle between them was 46° .

I. PRELIMINARY EXPERIMENT

In a preliminary experiment sixteen coniferous woods from the collection of the Imperial Forestry Institute, Oxford, were examined, the samples chosen being about $10 \times 10 \times 40$ mm. in size, and of apparently normal, mature heart wood of average ring width. The



Text-fig. 4. Diagram of specimen tube with wood sample and salt, as in preliminary experiment described in this paper.

squared and sanded blocks were enclosed in an equal number of glass specimen tubes fitted with airtight waxed corks, and laid horizontally on a wooden rack, with an equal quantity of a chosen moistened salt (see p. 436) in each, the wood samples being held clear of the salt by pins through the corks (see Text-fig. 4). Longitudinal sections of the same species were examined microscopically for determination of

the average inclination of the "seasoning slits" in their tracheide walls.

Duplicate specimens were also examined, and care was taken to see that the salts did not dry out nor actual condensation take place on the wood, the tubes being kept at a fairly uniform temperature of about 18° C. The samples were rotated in their tubes daily by means of the pin, projecting through each cork, and weekly measurements of mean length were made, *in situ*, with a travelling microscope provided with vernier scale, the humidity of the air in the tubes being reduced in progressive steps from 95 to 1 per cent R.H. by means of the following salts.

Pure recrystallized chemicals were used, and the same series was later employed in the main investigation. Their reliability for the given purpose was tested beforehand by Mr J. F. Martley, who was at that time making a special study of hygroscopicity and moisture movement in woods at the Forest Products Laboratory.

Name of salt	Aqueous condition	Approx. relative humidity at 30° C. %
Sodium carbonate	Just moistened	95
Sodium chloride	" "	77
Calcium nitrate	" "	57
Calcium chloride	" "	35
Oxalic acid	$\frac{1}{2}$ normal and $\frac{1}{2}$ anhydrous	10
Phosphorus pentoxide	Anhydrous	1

The result of the preliminary experiment was to indicate that a fair general correlation, viz. $+0.734 \pm 0.079$, existed between the angle θ and percentage longitudinal shrinkage, except for three specimens in which the former value was uncertain (see Table I). Correlation of sine θ^0 with percentage longitudinal shrinkage gave a slightly smaller result, viz. $+0.709 \pm 0.084$.

II. MAIN EXPERIMENT

Measurement of longitudinal shrinkage

Samples were again selected from apparently normal heart wood of thirteen conifers in the I.F.I., Oxford collection, having annual rings of average width about $\frac{1}{8} - \frac{1}{12}$ in. Four thin slips, mutually adjacent, and including parts of the same growth rings, were then cleft from the former and squared and sanded to a size of about $50 \times 5 \times 3$ mm. Straight-grained wood was chosen, and only three

TABLE I. Results of preliminary experiment

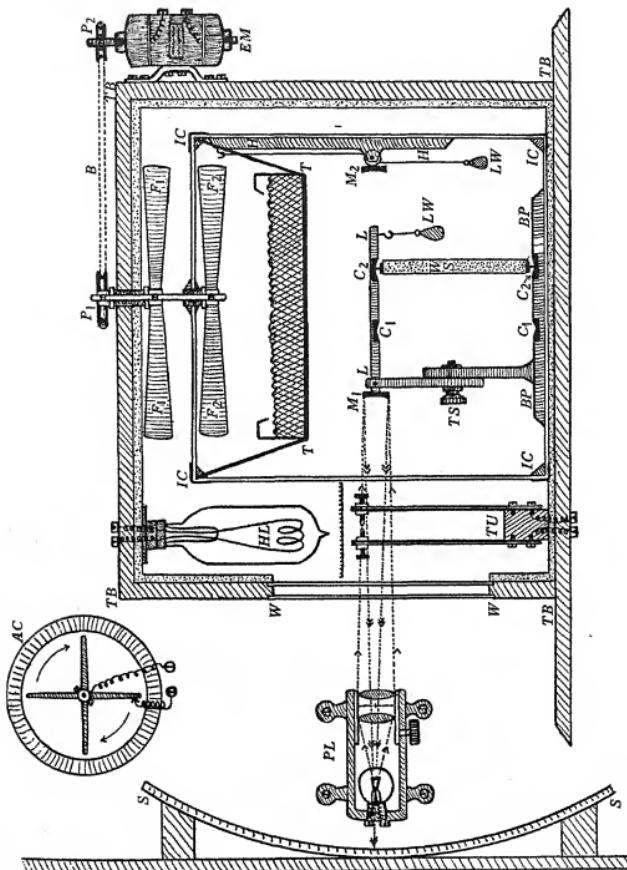
Wood species	Mean inclination to vertical (and range) of cracks in tracheide walls	% shrinkage (95-1 % R.H.)
<i>Abies pectinata</i>	20° (18-28°)	0.58
<i>Agathis australis*</i>	45° (40-50°)	1.72
<i>Chamaecyparis Lawsoniana*</i>	22° (17-26°)	0.61
<i>Cupressus sempervirens</i>	14° (12-19°)	1.24
<i>Juniperus procera</i>	32° (17-33°)	0.78
<i>J. virginiana</i>	23° (28-37°)	0.27
<i>Larix Europea</i>	15° (5-25°)	0.59
<i>Libocedrus decurrens</i>	45° (37-53°)	2.75
<i>Pinus palustris</i>	9° (6-12°)	0.28
<i>P. resinosa</i>	27° (21-35°)	0.68
<i>P. strobus</i>	26° (23-31°)	0.77
<i>P. sylvestris*</i>	27° (20-31°)	0.55
<i>Podocarpus elongata</i>	32° (23-38°)	0.82
<i>P. gracilior*</i>	30° (25-33°)	0.95
<i>Pseudotsuga Douglasii</i>	15° (13-20°)	0.26
<i>Tsuga heterophylla</i>	26° (20-30°)	0.72

* Compression wood? Compare with values in Table II.

sticks out of the thirty employed actually suffered appreciable warping during treatment, and had to be discarded; though possible warping is certainly a serious drawback with thin pieces of wood. Thicker samples, however, considerably increase the time factor. Even with such thin slips, the drying period of each sample was 2-4 weeks, and time was lacking to complete all the species that had been chosen.

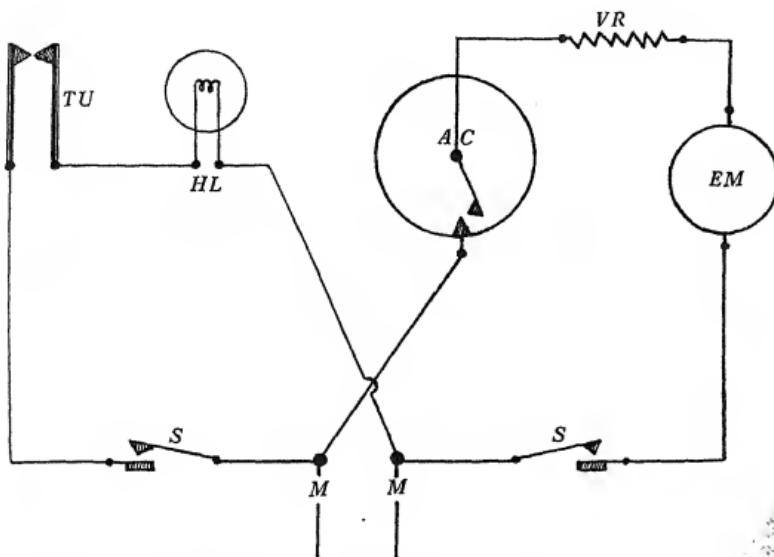
All samples were squared parallel to the grain and the growth rings; and each one included both summer and spring wood, symmetrically placed: either spring wood in the middle, between two zones of summer wood, or conversely. Warping due to unequal shrinkage of the two components was thus practically avoided (as tests against a straight-edge proved), while it was hoped that an average value for spring and summer wood would also thus be obtained. Presumably, however, the component with the smaller shrinkage shackled the other; and had spring or summer wood alone been employed for both shrinkage and slit angle measurements, yet better correlations would probably have resulted. In fact, judging by the steeper angle of the slits obtaining in the summer tracheides as compared with those of the spring wood (see below), longitudinal shrinkage should, *ex hypothesi*, have been smaller in the former and greater in the latter.

Three or four of the prepared samples of each of the selected species were measured for percentage longitudinal shrinkage with a



Text-fig. 5. Abbreviations: *SS*, curved vertical scale, divided in mm.; *PL*, projector light, fitted with cross wire; *AC*, modified alarm clock, to switch on fan motor every $\frac{1}{4}$ hour; *HL*, carbon filament heating lamp, operated by thermostat unit (*TU*); *TU*, bi-metallic strip thermostat unit, operating heating circuit, and protected from direct rays of the latter by screen, shown as dotted line; *WW*, double glass window in side of thermostat box, for passage of light rays between projector, mirror and scale; *P₁-P₂*, pulley wheels on fan and motor connected by rubber band (*B*); *F₁-F₂*, fans (driven by electric motor) inside thermostat box and inner sealed chamber, mounted on a single shaft; *TT*, enamelled tray carrying moist salts for R.H. control, mounted on wall inside sealed chamber; *LL*, lever arm of

specially constructed apparatus, about to be described. For the actual percentage shrinkages of the second batch of specimens, between 95 and 1 per cent fibre saturation, see p. 442, Table II.



Text-fig. 6. Wiring diagram of electrical connexions to thermostatic chamber, etc. Abbreviations: MM, electric mains terminals (100 V. D.C.); SS, mains switches; TU, thermostat control unit; HL, carbon heating lamp; AC, alarm clock, controlling EM; VR, rheostat, controlling EM; EM, electric motor, working fans.

Correlations between the former values and those for slit angle (θ) will be discussed in a subsequent section.

The special apparatus for exact measurement of longitudinal dimensional changes, which was designed by the writer at the suggestion of Mr J. F. Martley, M.Sc., may be outlined as follows. A diagram and further details are given in Text-figs. 5 and 6.

Text-fig. 5. Abbreviations (contd.)

measuring instrument, pivoted at left-hand end, and supported by wood sample at right-hand end; TS, thumb screw for adjusting height of lever arm above base plate (BP); M_1 , reflecting mirror on end of lever arm; WS, wood sample under measurement, supported by drawing pins at either end, cemented on with shellac; C_1-C_2 , ruby cups, inset into lever arm and base-plate, for support of wood sample; LW, small lead weight; M_2 , reflecting mirror of hygrostat; HH, hair hygrostat; TB, thermostat box, insulated with asbestos; IC, inner glass chamber, sealed at corners with insulation tape and "Plasticene"; EM, electric motor, operated by alarm clock, and driving two fans.

(1) A sealed glass hygrostatic chamber. This contained the wood specimen under examination, held in a vertical position between a fixed base plate and a small lever arm, carrying a mirror at one end; from which a beam of light was reflected on to a distant scale, so as to magnify small movements of the lever arm with dimensional changes in the wood sample. Also a hair hygrometer, an electric fan to stir the air, a dish of moist salts for R.H. control, and a thermometer.

(2) An insulated thermostat box, enclosing (1). This was fitted with an electric carbon-filament heating lamp, a second stirring fan, a bi-metallic thermostatic control unit (to make and break the heating circuit automatically at a chosen temperature), and a double-walled glass inspection door. There was also a side panel, for transmission of the two light beams from the hygrometer and lever mirrors respectively.

(3) External to (1) and (2) were a fixed galvanometer lamp and a distant curved mm. scale, concentric with the lever-arm mirror. The lamp was situated so as to throw two light spots and cross wire images, from the hygrometer and lever-arm mirrors respectively, on to opposite edges of the mm. scale.

(4) A small (mains) electric motor, to drive the fans in the thermostat case and inner chamber. This was switched on automatically, for 1 min. every quarter of an hour, by the minute hand of a modified alarm clock with four spring contacts.

The hygrometer was used simply to observe when the relative humidity of the air in the sealed chamber had become constant. It was, however, also roughly calibrated. The thermostat box was well insulated with asbestos sheeting, and tests at various points of the inner chamber (which was maintained during experiment at about 30° C.) showed a temperature range not exceeding $\pm 0.5^\circ \text{C}$. The lever instrument for recording longitudinal changes in the wood samples is shown in section in Text-fig. 5. It was made of brass, lacquered and greased, and consisted of a vertical pillar of adjustable length, firmly welded to a circular base plate, and carrying a pivoted horizontal arm at its upper end. To the pivoted end of the arm was fastened a small galvanometer mirror, as already described; while from the other, free, end was suspended a 20 g. lead weight. Finally, two pairs of minute conical ruby cups were inset, vertically above one another, in the upper side of the base plate and the under side of the lever arm respectively. These cups held in position the wood sample to be measured, by means of two flat-headed brass drawing

pins, tightly cemented with shellac to the two squared ends of the sample. Considerable movement of the light spot on the scale resulted from very minute alterations in the length of any wood sample under test, magnifications of $\times 168$ and $\times 42$ linear being given, according to which pair of ruby cups was used. Actually, the higher magnification was employed throughout the present experiment, and the samples were about 50 mm. long.

Whenever it was necessary to change the moist salts in the sealed chamber, the latter was opened for as short a while as possible, the front face being hinged at the bottom, the whole hermetically sealed with "Plasticene", and the dual fan shaft passed into the two chambers through well-greased stuffing boxes.

With this apparatus the percentage shrinkages of the selected wood specimens were successively measured with a high degree of accuracy.

Measurement of mean slit inclination (angle θ)

In determining slit inclination, radial longitudinal sections were employed, and a tangent to the midpoint of a slit or stria, as seen in surface view on an undamaged tracheide wall, was taken to represent the mean inclination of the former relative to the middle lamellae of the longitudinal walls. A magnification about $\times 750$ linear by a good compound microscope, provided with a special rotating eyepiece having a cross-thread and circular scale of degrees, was employed, and about three hundred slits were measured, in three traverses of each section. The sections were cut from the same samples as the specimens used for the shrinkage measurements.

Judging by repetitions of measurements made on the same tracheides, the maximum experimental error was about $\pm 2^\circ$ for an individual slit, but much less than that, of course, for the mean values for each species.

As will be seen from Tables I to IV, there was a considerable range of variation in the slope of the slits from tracheide to tracheide in any given species and sample; and the advisability of using the same identical samples for both slit angle and shrinkage measurements was subsequently realized. Actually, however, the sections for measurement of the slope of the fibrillar spirals were cut from wood immediately subjacent to, and in the same vertical increment cylinder as, the shrinkage samples, as the latter were too thin to section conveniently. It is feared that this small discrepancy may have lowered the correlation values.

TABLE II. Results of main experiment

Wood species	Mean inclination to vertical (angle θ) of cracks in tracheide walls, and range	% shrinkage (95-1 % R.H.)	Mean % shrinkage	
			of samples	I-3
<i>Agathis australis</i>	25° (11-35°)	(1.280)* 0.328 0.273 0.252		0.28
<i>Chamaecyparis</i> <i>Lawsoniana</i>	8° (3-17°)	0.228 0.248 0.318		0.27
<i>Juniperus procera</i>	28° (19-35°)	1.423 1.480 1.370		1.42
<i>Larix Europea</i>	16° (5-28°)	0.283 0.190 0.613		0.36
<i>Pinus palustris</i>	10° (3-19°)	0.263 0.323 0.377		0.32
<i>P. resinosa</i>	25° (8-37°)	0.410 0.788 0.368		0.52
<i>P. strobus</i>	27° (20-35°)	0.566 0.781 0.864		0.73
<i>P. sylvestris</i>	17° (5-28°)	0.364 0.475 0.337		0.39
<i>Podocarpus elongata</i>	30° (18-40°)	0.563 0.472 0.470		0.50
<i>P. gracilior</i>	19° (5-35°)		Not determined	
<i>P. Milanjianus</i>	24° (10-36°)		Not determined	
<i>P. Thunbergii</i>	13° (3-35°)		Not determined	
<i>Tsuga heterophylla</i>	26° (8-48°)	0.174 0.167 0.143		0.16

* First sample evidently abnormal. Possibly compression wood.

As instance of variability of the mean slit inclination in a single species: in one specimen of normal wood of *Abies pectinata*, the mean value for angle θ was found to be 20° (range 18-28°), in a second specimen 33.5° (range 32-35°), and in a third specimen—of red compression wood incidentally—it was 41.2° (range 34-52°). Hence for this species, at least, the observation that the fibrils or micellae

tend to run more obliquely in compression than in normal wood was, apparently, confirmed.

Unfortunately, it was not known for certain whether the specimens listed in Table II contained either compression or tension wood, but, judging by colour and ring width, they were all of normal growth. Nor did time permit of further detailed comparisons of spring and summer, tension and compression wood being undertaken with regard to shrinkage behaviour. Note, therefore, that the values for θ given in Tables I, II and IV are only representative of the selected samples chosen, irrespective of local growth characteristics; whereas the longitudinal shrinkage values were hoped also to approximate to the means of the spring and summer wood zones conjointly, and to average out any local peculiarities.

The relationship between angle θ and tracheide width was also briefly considered, and will be dealt with separately below.

Discussion of results

The results of the second experiment, shown in Table II, gave the moderate positive correlation of $+0.540 \pm 0.015$ between mean slit angle θ (assumed equivalent to fibrillar slope) and percentage longitudinal shrinkage; viz. shrinkage between hygroscopic equilibrium in a 95 per cent saturated atmosphere and one of 1 per cent saturation, at 30°C ., expressed as a percentage of the maximum length of the samples in the 95 per cent saturated atmosphere.

It will be seen from Table II that the degree of positive correlation was considerably lowered by *Tsuga heterophylla*, in which the percentage shrinkage, though fairly constant for the three samples measured, seemed abnormally low, *ex hypothesi*, as compared with the corresponding mean value of θ , namely, 26° . Omitting this wood, a positive correlation over $+0.6$ would be given by the remainder.

Although the correlation obtained from the second set of data was not quite so good as that of the preliminary experiment (viz. $+0.734 \pm 0.079$), a significant positive correlation between the two features is confirmed; especially considering the great variability of fibrillar inclination observed within wood of a single species and the fact that identically the same samples were not used for the two sets of measurements, as already explained. Moreover, the relative hygroscopicity of the woods may have varied from sample to sample; and in endeavouring to shorten the time taken over the shrinkage measurements, by reducing the thickness of the specimens, it is

possible that an extra risk of warping of some of the thin slips was introduced. Warping should have appreciably increased the apparent shrinkage values; and, in fact, the second sample of *Pinus resinosa*, which was the only sample appreciably warped as judged by a straight-edge, actually showed a greater apparent shrinkage than the other two samples of the same wood. On the other hand the first sample of *Agathis australis*, which also gave an extra large shrinkage compared with the other three samples, was not appreciably warped. Sample I, therefore, was probably abnormal, resembling compression wood in character. (Cf. Jaccard & Frey, 1928.)

III. SECONDARY OBSERVATIONS

Slit inclination and tracheide width

During the measurement of angle θ for slits and striae in the tracheide walls, it became evident that θ tended to vary progressively, along with tracheide width, across each annual ring. This aspect of the matter was, therefore, followed up in some detail, as an extension of the main experiment, and my observations may be summarized as follows:

(1) In all the woods examined, there certainly appeared to be a definite relation between the type of wood—whether spring or summer wood—and the mean angle (θ^o) of the slits (see Pl. VII, fig. 1).

Thus, in the following woods, in which spring and summer wood were well differentiated, distinctive values were obtained for the two zones. (Means and ranges stated.)

Wood species	Spring wood	Summer wood
<i>Juniperus procera</i>	29·5° (20–38°)	25·3° (19–34°)
<i>Podocarpus elongata</i>	31·0° (19–40°)	28·0° (18–34°)
<i>Pinus resinosa</i>	26·0° (10–35°)	22·0° (8–35°)
<i>P. strobus</i>	29·5° (22–35°)	25·0° (20–28°)
<i>Podocarpus Thunbergii</i>	19·0° (10–35°)	8·0° (3–18°)
<i>Tsuga heterophylla</i>	34·0° (24–38°)	17·0° (8–35°)

(2) It was further realized that, apart from the zonal factor, angle θ was to a large extent dependent upon the width of the individual tracheide. This was apparent at a glance in many sections, and was shown to be so by the fact that a tangential longitudinal section gave values for θ varying considerably: i.e. in the same age plane, whether in spring or summer wood.

For example, on the one hand, in *Podocarpus Thunbergii* some narrow elements were encountered in the midst of the spring wood,

for which the angle θ was only $10\text{--}15^\circ$, as compared with the $17\text{--}24^\circ$ inclination of the slits in their broader neighbours, the widths of the narrow and broad tracheides being about as $2/3$. In *Pinus resinosa*, a tangential section taken at the junction of the spring and summer zones gave a mean value of 32° , and a range of variation of $26\text{--}37^\circ$ for θ . Compare this with the values given above for the spring and summer wood separately. The range of variation, though smaller than that obtained from a radial section (i.e. *across* the growth ring), is still considerable, indicating that the slope of the micellar spirals may be different in different cambial initials.

(3) In order to determine the degree of correlation between slit angle (θ) and the radial tracheide width, the widths of all the tracheides at a given level across a single annual ring were successively measured upon a radial section of *Tsuga heterophylla*. The width of the lumina and single wall thickness were also determined (see Tables III A, B).

The following correlations were thereby obtained:

$$\text{Slit angle } (\theta)/\text{tracheide width } +0.902 \pm 0.022$$

$$\text{Slit angle } (\theta)/\text{width of lumen } +0.914 \pm 0.020$$

$$\text{Slit angle } (\theta)/\text{thickness of wall } +0.224 \pm 0.113$$

N.B. These values—based upon the summer wood only, owing to lack of slits, etc., in the spring tracheide walls—show that an exceedingly good positive relationship connected tracheide width, and, still better, *width of lumen*, with the inclination of the fibrils in the cell walls. On the other hand, there is no evident dependence of the angle θ upon cell-wall thickness.

(4) It was further observed, both in *Tsuga* sp. and other woods, that the angle θ for a narrow spring-wood tracheide might be approximately equal to that of a summer tracheide of the same width, or that of a wide summer tracheide to an equally broad spring element.

Tracheide width, therefore, appears to be chiefly responsible for the inclination of the fibrils being less steep, on the average, in the spring than in the summer wood of a tree.

N.B. These observations confirm the conclusions of Jaccard & Frey (1928) that the inclination of the micellar series, or fibrils, is dependent upon local developmental processes—and hence, presumably, upon relative growth rate.

(5) The next thing to determine was whether the angle θ was decided by tracheide width alone, or whether other factors must also be taken into account.

TABLE III. A. *Summer-wood tracheides of Tsuga heterophylla*
 (Individually measured across a single annual ring, in natural
 order of occurrence—means of three traversals)

Total width of tracheide (approx.)	Width of lumen (approx.)	Thickness of tracheide wall (approx.)	Inclination of slits to vertical (angle θ)
49	42	3.5	35°
42	35	3.5	32°
42	35	3.5	34°
42	35	3.5	31°
42	28	7.0	30°
42	21	10.5	27°
35	18	8.5	15°
31	18	6.5	18°
31	14	8.5	15°
25	11	7.0	14°
28	14	7.0	8°
28	14	7.0	10°
28	14	7.0	12°
25	11	7.0	12°
28	14	7.0	9°
28	18	7.0	12°
28	14	5.0	11°
25	11	7.0	14°
28	14	7.0	14°
28	14	7.0	14°
28	14	7.0	18°
25	11	7.0	19°
28	14	7.0	15°
29	18	7.0	15°
28	18	5.5	18°
24	14	5.0	14°
29	18	5.0	15°
20	7	6.5	14°
21	11	5.0	12°
16	6	5.0	11°
11	3	4.0	10°
Means 29	17	6.3	7°
			17°

B. *Spring-wood tracheides of Tsuga heterophylla*

Total width of tracheide (approx.)	Width of lumen (approx.)	Thickness of tracheide wall (approx.)	Inclination of slits to vertical (angle θ)	
			In clear wall	In pits
53	46	3.5	34°	58°
53	47	3.0	27°	70°
56	50	3.0	45°	77°
53	47	3.0	—	69°
53	47	3.0	42°	74°
53	47	3.0	25°	68°
53	48	2.5	37°	72°
56	52	2.0	—	75°
53	48	2.5	—	70°
49	44	2.5	—	66°
49	44	2.5	—	66°
42	38	2.0	—	64°
42	37	2.5	40°	68°
42	37	2.5	—	65°
42	37	2.5	24°	66°
45	38	3.5	34°	66°
45	38	3.5	38°	66°
Means 49	44	2.8	35°	68°

In the first place, it was evident that there were many exceptions to the general rule, even with a single sample or growth ring; the positive correlation between angle θ and tracheide or lumen width being merely a generalization from the majority of instances. For example, wide tracheides were often observed with steeper cracks than much narrower ones near by, and conversely.

Second, in the portions of tracheides in contact with rays, the slope of the cracks was generally steeper than in their free portions; thus simulating summer-wood elements, and suggestive of some sort of binding action of the ray preventing the free expansion of the growing tracheides. Thus, in the spring wood of *Tsuga heterophylla*, the mean angle θ in contact with the rays was found to be 13° (range $1-22^\circ$), as compared with 34° ($22-48^\circ$) in the "free" portions of the tracheide walls. The former values agreed more nearly with that found for the summer-wood elements in the same species; namely, 17° (range $8-35^\circ$). The mean inclination to the vertical of the cracks through the pits was also reduced in the same way.

N.B. Such ray-affected regions were not included in the general measurements shown in Tables I-III, though their omission may, conceivably, have slightly affected the mean values for θ , and hence the degree of correlation between θ and percentage longitudinal shrinkage.

In the third place, it was observed that tracheides of equal width in different species did not necessarily give the value for angle θ ; while it would appear that woods having a more or less coincident range of slit angle may (e.g. *Podocarpus elongata* and *Juniperus procera*) or may not (e.g. *Tsuga heterophylla* and *Pinus resinosa*) each exhibit equivalent θ values for tracheides of any given width.

Thus, the following data were obtained from measurements of θ in my samples of the five woods listed; showing that, for tracheides of a given width, angle θ might vary considerably from species to species—as well, to some extent, as within a given species or sample (see also Table IV):

Means and ranges of fibrillar slope (θ)
in tracheides of mean width =

Tree species	$14\ \mu$	$28\ \mu$
<i>Juniperus procera</i>	25.0° ($23-28^\circ$)	32.5° ($30-35^\circ$)
<i>Podocarpus elongata</i>	22.5° ($20-25^\circ$)	28.0° ($25-35^\circ$)
<i>Tsuga heterophylla</i>	10.0° ($8-15^\circ$)	14.0° ($8-17^\circ$)
<i>Pinus resinosa</i>	15.0° ($14-16^\circ$)	27.5° ($25-30^\circ$)
<i>P. strobus</i>	18.0° ($11-27^\circ$)	23.5° ($20-27^\circ$)

Range of θ for the lot $8-28^\circ$ $8-35^\circ$

TABLE IV. Relationship between slit angle (θ) and tracheide width

Tree species	Observed range of angle θ	Observed range of tracheide width μ
<i>Agathis australis</i>	11-35°	22-40
<i>Chamaecyparis Lawsoniana</i>	3-17°	11-46
<i>Juniperus procera</i>	19-35°	8-35
<i>Larix Europea</i>	5-28°	13-78
<i>Pinus palustris</i>	3-19°	13-73
<i>P. resinosa</i>	8-37°	11-62
<i>P. strobus</i>	20-35°	8-65
<i>P. sylvestris</i>	5-28°	11-54
<i>Podocarpus elongata</i>	18-40°	13-43
<i>P. gracilior</i>	5-35°	19-51
<i>P. Milanjanus</i>	10-36°	24-51
<i>P. Thunbergii</i>	3-35°	16-46
<i>Tsuga heterophylla</i>	8-48°	11-59
Range of variation of the lot	3-48°	8-78

Note. The above values refer to the radial sections of one sample only of each wood—those employed in the present experiment.

Note. These data do not represent final specific values, being derived only from single radial sections. They show, however, that angle θ is not dependent upon tracheide width alone, considered interspecifically, but possibly also on some specific molecular patterning.

It was sometimes observed that the inclination of the slits was almost as steep in the first two or three (wide-lumened) tracheides of the spring wood as in the last few (narrow-lumened) tracheides of the summer wood, although this was not a constant feature.

Note, in this connexion, that from observations on the spring and summer wood of *Tsuga heterophylla* and *Pinus resinosa*, it appeared that the degree of correlation between angle θ and tracheide width was not so definite in the spring as in the summer wood.

(6) In a single tracheide there was often some variation in angle θ from part to part. For example, in a tracheide of *Juniperus procera*, from 21-30°, mean 26.6°. Such variation sometimes, though not always, was associated with variations in tracheide width.

(7) The cracks and striae upon the tracheide walls were often seen criss-crossed, the values for the two sets being found equal. Focusing up and down with a high power of the microscope always showed that the two sets of cracks or markings originated in separate planes, presumably in distinct layers of the cell wall, the direction of the spirals in a given element always being the same in a given plane—whether clock- or anti-clockwise (see Pl. VII, fig. 3). The same

remarks apply to the slit-shaped (often cracked) pit openings. Unlike the cracks in the plain wall, however, the slope of the slit-like openings of the bordered pits was not always equal on the two sides. Thus, in *Podocarpus Thunbergii*, the angle on one side had a mean value of 15° , on the other 37° to the vertical, for one tracheide pair. In another pair the two angles were 29 and 38° respectively.

(8) The relation of the pit openings (where non-circular) to the slits in the plain tracheide wall is both interesting and hard to generalize. Unfortunately, many investigators have employed pit openings to determine mean fibrillar slope; though that was evidently unwise. (Cf. Haberlandt, 1914, p. 550.)

Judging from the woods examined, I should say that, in the dense summer wood, the slit-shaped or oval openings of ray parenchyma pits, as well as the oblique pit openings of certain bordered tracheide pits, generally followed the inclination of the cracks and striae of the plain wall (see Pl. VII, fig. 1). In spring wood, however, such was by no means always the case. Thus, in the late spring wood of *Tsuga heterophylla* some of the bordered pits were found to have cracks across their openings at about 45 – 90° inclination to the long axis of the tracheide, as compared with only 10 – 20° for the cracks in the plain walls of the same cells. Similarly in *Pinus resinosa*, though occasionally agreeing with the ordinary slit angle, the inclination of the pit cracks was very variable—even reaching 60° to the vertical.

In *Pinus strobus*, the inclination of the slit-shaped simple pits of the ray parenchyma, as well as the openings of the bordered pits themselves in the late summer wood, coincided with that of the plain wall cracks. Not so, however, every pit crack or pit opening in the spring wood.

So too, in *Agathis australis*, the pit openings and wall cracks either ran parallel or approximately coincided; and in *Podocarpus Thunbergii* the pit openings followed the fibrils throughout both spring and summer wood (see Pl. VII, fig. 4).

In *P. gracilior*, however, there was the same sort of contrast, in the spring wood, as in *Tsuga heterophylla*: 40 – 50° for pit cracks or openings, as compared with 15 – 35° (mean 19°) for the plain cell walls.

Very occasionally, irregular cracks were seen around the openings of bordered pits that more nearly fitted a theory of concentric structure. Their rarity and irregularity, however, suggested that they were artifacts rather than an indication of molecular arrangement.

It was noticed that, even where the direction of a pit crack approximately coincided with that of a conjoined crack in the plain

wall, the portion of the crack running through the pit nearly always ran slightly more horizontal than the rest.

It was concluded, therefore, that the structure of the cell wall, so far as the direction of the micellar series was concerned, was often modified in the region of a pit; though the most common direction of cracking or elongation did not suggest any concentric structure in the woods examined (cf. Ritter, 1928).

(9) In the sections of *Podocarpus Thunbergii*, which had been rather lightly stained with safranine, it was observed that the cell walls had stained more densely on either side of a crack than elsewhere for a distance of some $1\text{--}4\mu$.

Moreover, it should be noted that, although the sections were dual stained with iron haematoxylin and safranine, it was the *lignin* stain that was primarily taken in the neighbourhood of the cracks—not the haematoxylin.

This behaviour is reminiscent of slip planes (see Robinson, 1921), where molecular disturbance, however, produces a definite *cellulose* reaction to microchemical tests, and the lines of dislocation show up brightly against a dark ground between crossed Nicol prisms. The use of a polariscope on the cracks in the present case gave a similar result (see Pl. VII, fig. 2 and Pl. VIII, fig. 2.)

(10) It should be mentioned that the radial walls of tracheides were generally more heavily cracked than the tangential ones, and tangential sections were used only as supplementary evidence in arriving at means and ranges for angle θ .

When present on the tangential walls, the inclination of the cracks and striae was found to be about the same as that of the cracks on the radial walls at a corresponding position in an annual ring.

Slip planes in Abies sp.

It is interesting to compare the inclination and nature of the so-called "slip planes", frequently met with in the cell walls of timber that has been subjected to considerable stress or strain, with the slope of the "seasoning cracks" in the same wood.

Robinson (1921) has already fully investigated this phenomenon, but the following observations upon a specimen of *Abies pectinata* are of interest in the present connexion. Otherwise, the observations of Robinson were corroborated (see Pl. VIII).

(1) The range and mean value for the inclination of the slip planes to the longitudinal tracheide walls were distinctly greater than those of the seasoning cracks in the same species: namely, 68°

(45–80°) for the former as compared with about 20° (18–28°) for the latter. Unfortunately, the same sample did not contain both types of failure, but the difference in θ is probably sufficiently great to be counted upon.

(2) In crossed slip cracks—of common occurrence—the slope of the two, or more, components was typically about equal, as in the case of seasoning slits.

(3) The inclination of the slip cracks in the walls of spring- and summer-wood tracheides was not appreciably different.

(4) Slip cracks were sometimes seen to be wavy, though normally straight.

(5) The tissue immediately adjoining each individual cleavage plane was seen, under the polarizer, to be in a state of molecular disturbance and stress; and the same narrow zone generally showed a modified staining reaction—as noted by Robinson and others. These two features are similar to those observed for the seasoning slits.

It is concluded from these observations that the micellar planes, or molecular patterns, which determined the direction of the slip cracks, were not coincident with those of the seasoning slits in the same wood species. At the same time, it is evident from the relative constancy of their inclination that there did exist determining factors of the same sort in the two instances, as also in the case of Kelaney's and Searle's approximately transverse "chemical sections" of fibres (1930); all such failures and cleavage planes, doubtless, being dependent upon the directions of the multiple molecular patterns suggested by X-ray analysis. (E.g. Sponsler & Dore, 1926–28.)

It might, perhaps, be supposed that the seemingly different slopes of the slip planes and seasoning cracks were due to the former being observed in a sectional and the latter in a surface view (cf. Plates VII and VIII). Reference to a helically coiled flat clock spring, taken as a model, will show that in cross-sectional, instead of surface, view cleavage planes such as the seasoning slits might appear more or less at right angles to the long axis of the helix (i.e. θ approximately 90°). Actual microscopic features, however, strongly suggested the non-coincidence of the two phenomena in question.

Moreover, had exactly similar molecular or micellar planes determined both types of failure, the mean slope of the slip planes also should have varied with tracheide width from spring to summer wood, which it did not do in the specimen examined.

Pit openings in common oak

As I had been working concurrently upon the wood structure of common European oak (*Quercus Robur* L. and *Q. sessiliflora* Salis.), and had often noticed a tendency to obliquity both in the alignment of the vessel and tracheide pits and their slit-like openings, it seemed worth while to make a more careful examination of these features in connexion with the present enquiry. Some four hundred sections from about twenty trees of diverse origin and growth type were, therefore, cursorily examined, with the results given below.

(1) A linear transverse or oblique arrangement of the pits on the walls of the conducting vessels did sometimes occur sporadically; but it was evidently an "accidental" rather than a specific feature, and was not even general within a single vessel element.

(2) The mean slope of the oblique slit-like openings of such pits, however, was relatively constant, and evidently depended upon some inherent feature of the cell wall, or molecular arrangement, as was assumed for the seasoning slits and slip cracks already described. No statistical data were collected.

(3) The narrower an oak tracheide, the steeper, generally, was the slope of the pit openings, as in the case of the conifer seasoning slits. (Actual cracks or striae were very rarely encountered in oak.) The inclination (θ) of the pit openings in the narrow tracheides varied between 25 and 45°, whereas the oval openings of the large spring vessels were invariably almost horizontal—indicating a similar correlation between cell width and fibrillar slope, as in the conifers. (Cf. Haberlandt, 1914, p. 550.)

SUMMARY

1. The correlation of percentage longitudinal shrinkage, over a given range of percentage fibre saturation, with the mean slope of certain spiral slits and microscopic fibrillae in the tracheide walls of sixteen species of coniferous woods, was confirmed.

2. A general corelationship between tracheide (and/or lumen) width and the slope of the vertical wall slits was found; though exceptions to this rule were not infrequently encountered. Wall thickness was not found to be correlated to fibrillar slope.

3. As corollary to (2), it was confirmed that the slope of the slits and fibrils was typically steeper in summer than in spring wood; varying more or less progressively across the annual ring.

4. It was observed that the slope of the slits in the tracheide walls

was typically steeper in contact with the medullary rays; and it was also confirmed that they were less steep in a sample of compression wood (*Abies pectinata*) than in normal wood.

5. In order to explain the frequently observed difference in fibrillar slope between different samples and species, for a given tracheide width, it was suggested that the slope of the fibrils in a given wood species might also depend upon specific basic constitution, as apart from the tracheide width, growth rate, etc. This would imply certain differences of physicochemical constitution, which might be determined by X-ray methods of analysis.

6. In several of the coniferous species examined, the inclination of the slit-shaped pit openings, both of the tracheides and of the ray parenchyma, was found approximately to coincide with that of the "seasoning slits" and fibrils in the plain walls; but the practice of employing pit openings and associated wall cracks to determine mean fibrillar slope was deprecated, as in some species, and in the spring wood especially, the rule did not hold good.

The slope of the pit openings in the tracheides and vessels of common oak were seen to follow a similar general rule, evidently in relation to fibrillar wall structure and cell diameter.

7. In a sample of *Abies* sp., the non-coincidence of slip cracks—due to mechanical strain—with the slits and fibrils under discussion was demonstrated. But similar changes in optical and staining qualities of the wood were observed in both instances of cell-wall failure, owing, presumably, to molecular disturbance and electro-chemical changes.

8. A special apparatus for the measurement of minute dimensional changes in wood (or other rigid hygroscopic bodies) with changes of relative atmospheric humidity was described. The design included thermostatic and hygrostatic control, and was largely automatic.

The investigation described was commenced in 1926 at the Forest Products Research Laboratory, Department of Scientific and Industrial Research, but was completed privately. The writer is indebted to Mr R. S. Pearson, F.L.S., late Director of the Laboratory, for permission to make use of the results of work carried out in the Laboratory in the preparation of the present paper, and to a former colleague, Mr J. F. Martley, M.Sc., for suggesting the investigation.

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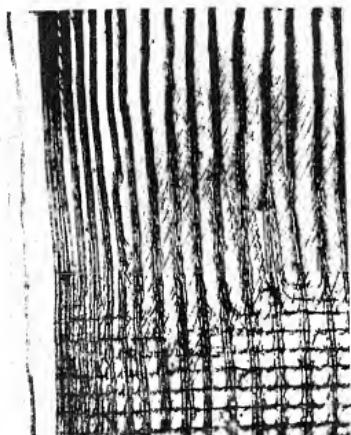
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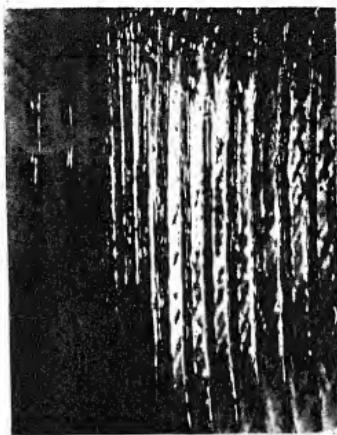
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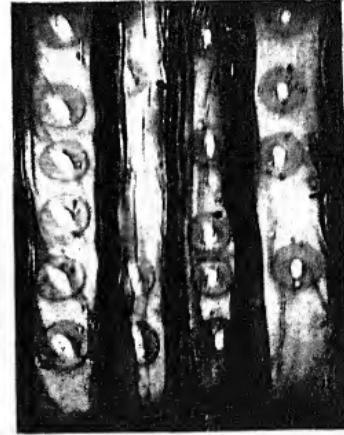
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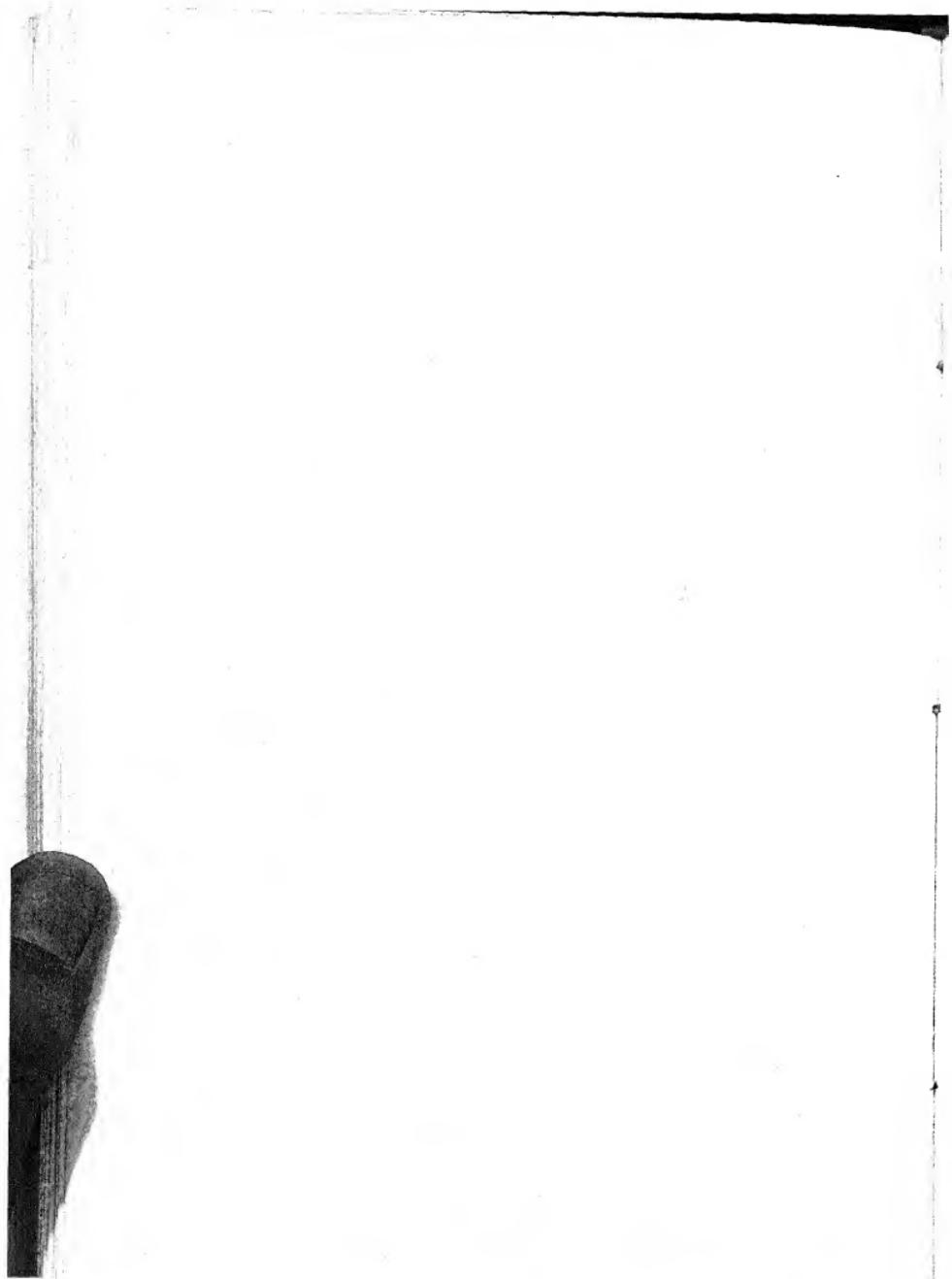


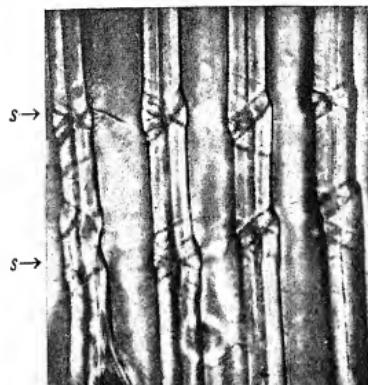
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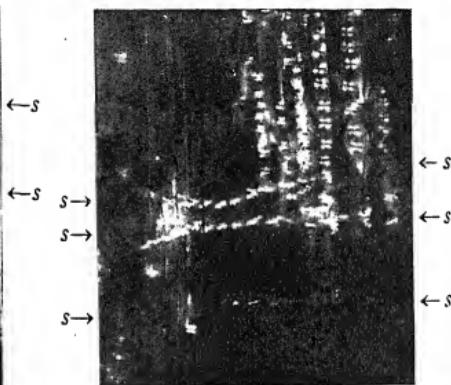
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MABY—MICELLAR STRUCTURE OF TRACHEIDES





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2



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4



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MABY—MICELLAR STRUCTURE OF TRACHEIDES



EXPLANATION OF PLATES VII AND VIII

PLATE VII

Fig. 1. Radial section through summer wood of *Pinus strobus*. ($\times 120$ lin.) Note: slits in radial walls, gradually becoming steeper as tracheides narrow, simple pits of ray parenchyma (in summer wood) conforming to inclination of seasoning slits.

Fig. 2. Radial section of *Pinus resinosa*, under polarized light, at position of right extinction of tracheide walls. ($\times 120$ lin.) Note local and general illumination of radial walls of summer-wood tracheides in region of the slits and striae.

Fig. 3. Radial section of *Pinus resinosa* showing crossed seasoning slits and striae. ($\times 500$ lin.) Note that the fainter X-hatchings pass under the alternate set, and that the slope gets steeper as one tracheide narrows upwards. The dark borders are due to refraction, showing dislocation of the wall adjoining each crack.

Fig. 4. Radial section of *Podocarpus Thunbergii*, showing bordered pits and seasoning cracks. ($\times 500$ lin.) Note the approximate agreement of the oval pit openings with the slope of the seasoning slits—sometimes running through them. (N.B. The slope of the pit openings and the wall slits did not always agree in this manner.)

PLATE VIII

Fig. 1. Slip cracks in tangential tracheide walls of a specimen of *Abies* sp. Unstained section, photographed by transmitted light. ss = slip planes. $\times 500$ lin.

Fig. 2. Ditto Fig. 1, but as seen in polarized light, showing illumination of the various dislocated parts, also of bordered pits. $\times 120$ lin.

Figs. 3-5. Sketches of slip cracks in a specimen of *Abies* sp. as seen by transmitted light in an unstained longitudinal section of the tracheides. $\times 1000$ lin.

SOME OBSERVATIONS ON THE PROBLEM OF VESSEL LENGTH DETERMINATION IN WOODY DICOTYLEDONS

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(With Plate IX and 4 figures in the text)

INTRODUCTION

A NUMBER of general statements as to the length of vessels in dicotyledonous trees will be found in the literature, but analysis usually shows the experimental basis upon which they are founded to be somewhat meagre and uncertain. In the course of the studies on wood anatomy which have been in progress during the last few years in the Botany Department in Leeds, injection experiments with Indian ink suspensions have frequently given indications of considerable vessel lengths. The observations have also directed particular attention to the very marked difference in length which appears to exist between the vessels of ring and diffuse porous types of wood. Dr Bruno Huber (1935) has recently pointed out that the conclusion reached in Leeds that the vessel lengths are much greater in ring porous types is in good agreement with his own observations upon the greater velocity of movement of water in these types also; his conclusion is based upon his experiments on the speed of propagation along the wood of a higher temperature from a point of local application on the surface of the wood. In the Leeds work the tree types with great vessel lengths have been found to be ones in which the resumption of cambial activity spreads extremely rapidly down the tree once it has been initiated in the buds, and a causal connexion between these two phenomena has been suggested (Priestley *et al.* 1933).

Whilst these injection experiments seem to place the great difference in vessel length between ring and diffuse porous types beyond all doubt, it was however noted in every case that the injection had apparently not reached the end of the vessel. So long as this is the case, there must remain some doubt as to the exact significance of this difference in the length of the injection, and the present report embodies the results of further investigations of the problem from two distinct angles.

In the first place an attempt has been made to recognize, from amongst the elements of the wood of ring and diffuse porous woods, the vessel segments which constitute the vessel "ends". Suitable maceration methods were employed and, after considerable difficulty, very definite results were obtained, so that vessel ends in both types can now be figured and, as would be expected, they are much more numerous in the diffuse porous woods. It has also been possible by a suitable modification of the injection technique to drive the injecting fluid right to the end of the vessel in certain cases and so to confirm the fact that the structural elements, regarded as vessel ends from the macerated material, have been correctly interpreted.

In the second place the older methods of injection have been more closely examined and new methods have been devised. The analysis of the experimental results has shown that the methods used in previous attempts are quite inadequate for the measurements of actual vessel lengths and has also served to emphasize the great difficulty of the problem involved in completely filling any vessel system with a recognizable suspension which will not pass the end walls.

HISTORICAL

Some of the earliest recorded results of the lengths of vessel injections are those given by Adler (1892), who used a method involving the production of a colloidal suspension of ferric hydroxide in the vessels, and Strasburger (1893) who used mercury injections, both of these also stated that the vessel length increased towards the periphery. Neither of these workers state whether the twigs were initially cut under water or the season of the year when the experiments were carried out; as will be seen later these are vital points of information for the proper interpretation of the results.

Ewart (1906) used a method of injection with mercury under pressure after first saturating the twig with water. As the mercury did not appear to inject the vessel right to the end, he tried mercury-sodium in place of mercury, and in a few such cases the injection seemed to have reached the end of the vessel, which, as seen in section, appeared pointed and tapering. In two later papers (1908, 1910) he states that, if branches are cut under mercury in June, July or August, the mercury is drawn into the wider vessels, as was also observed by von Höhnel (1879-81). The latter explained this as due to the formation of Helmholtz layers by the mercury in the vessels, the friction of the layers limiting the length of the injection, which is

dependent on the diameter of the vessel. The only wide vessel type injected by Ewart (1906) was wych elm (*Ulmus scabra* Mill), and this gave an injection length twice that obtained for narrow vessel types.

Comparison of these results of Ewart and those of Farmer (1919), who also used mercury, with the results obtained for trees of the same species by workers in Leeds using Indian ink suspensions, shows that much longer injections are obtained by the latter method, so that the mercury results are probably far short of true vessel lengths. There is also considerable doubt as to the reliability of the "vessel ends" seen by Ewart by sectioning methods. One of his figures on p. 352 (1908) shows what he took to be a vessel termination in *Wistaria*. This is illustrated as a transverse plate, whilst all the undoubtedly vessel ends in the present investigation, including those of *Wistaria*, have proved to be of a tapering form.

Bennett *et al.* (1927) describe a method of sap displacement from woody twigs and at the same time deduce certain facts regarding vessel lengths. They state that when gas under pressure is applied to one end of a twig, the sap in it will be driven forward, passing any cross-walls in the tracheae, until the process is stopped when the gas reaches the first cross-wall in its path; a portion of the twig is then cut off and the procedure is repeated until the sap is finally forced out of the twig. They omit to take into their considerations, however, the fact that many of the tracheae, especially those in inner annual rings, contain some air. The tracheae consist of longitudinally seriated systems of vessels (or tracheids) and, when the gas pressure is applied, the air bubble in each one of these will move forward; as soon as the air bubble in the shortest vessel of a system reaches a cross-wall, the movement forward of the sap in the whole length of this particular file of vessels will come to a standstill and no more sap will move forward in it until this particular shortest vessel has been cut away. This is probably the explanation of the extremely poor yield of sap obtained in the cases of pine and redwood. This argument also suggests that in experiments on displacement of sap by liquids, when the displacing fluid appears early in the expressed liquid, this does not prove that the particular length of twig contains open vessels as it might equally well be due to the fact that some of the vessels contain very little air.

This brief survey of earlier attempts to determine vessel lengths indicates that the initial state of the vessel contents were not taken into consideration, and this is obviously a point of vital importance

when aqueous fluids or mercury are used for the injections. Moreover, the methods of injection involved the use of either mercury or colloidal suspensions, and it will be shown later that the latter are not satisfactory owing to blocking effects. After injection, either no attempt was made to show that the fluid had reached the end of the vessel, or in other cases this was tested by sectioning, a method which is by no means reliable.

IDENTIFICATION OF VESSEL ENDS FROM MACERATED MATERIAL

The method of maceration was to place shavings of the wood in 5 per cent chromic acid in a vacuum extractor for 12-24 hours, or longer if it proved necessary, until sufficiently macerated. The material was then washed in water until free of all chromic acid and subsequently stained in glychaemalum, which was prepared from haematein obtained from haematoxylin by Mayer's method. After washing free from excess stain the material could be kept in 90 per cent alcohol. For microscopic examination it was mounted in pure glycerine and, where necessary, the mounts were made more permanent by ringing with Canada balsam (neutral).

The glycerine mounts have the advantage that an element which has the appearance of a vessel termination from one viewpoint may be rolled over and examined from all angles so that no perforations are overlooked.

Search for vessel terminations was first made in the tips of the current year's extension growth, and the success met with here led on to further search in the twigs and finally in the trunk. Both ring and diffuse porous types were examined.

DIFFUSE POROUS WOODS

(a) *Acer Pseudo-Platanus L.*

Vessel terminations were identified in material from the top of the current year's extension growth, from a 4-year-old twig and from the trunk, and in each case closely resembled those figured for corresponding situations in *Aesculus*. As is also the case with normal vessel segments, the terminations from the trunk material were larger in all dimensions than those from the 4-year-old twig and, also, those from the trunk were tapered to a blunter point. The terminations from the current year's extension growth were similar to the normal vessel segments except for the absence of a perforation at one end.

(b) *Aesculus Hippocastanum L.*

Material was examined from similar situations as in *Acer*, except that a 2-year-old twig was used in place of the 4-year-old. Terminations from the various positions are illustrated in Pl. IX, figs. 1, 2 and 3. In both *Acer* and *Aesculus* elements of the types illustrated were of relatively frequent occurrence.

RING POROUS WOODS

(a) *Fraxinus elatior L.*

From the spring wood of the trunk a vessel segment of the type shown in Pl. IX, fig. 4, was found. The pattern of the pitting and the position of the two perforations make it almost certain that this element formed a common termination to two vessels situated side by side, a very frequent grouping of vessels in this wood. The presence of this type of termination and the light it throws upon the nature of the associated vessel system suggests a probable explanation of puzzling observations made during the injection of the spring wood of *Fraxinus* with Indian ink in Leeds. It was occasionally observed that when the cut was made, the ink would rush up one vessel and after a short time it would be seen to rush down an adjacent vessel in the opposite direction. At the time the phenomenon seemed quite inexplicable, but coupling it with the system postulated on the grounds of the presence of this type of vessel end, common to two vessels, it seems that the ink travelled up one vessel and, on reaching this peculiar type of segment, had its course diverted down the vessel adjacent to the one already injected. This conception is further substantiated by the fact that the large spring vessels in *Fraxinus* are frequently arranged in radial pairs. It would also explain why calculations of the length of spring vessels in *Fraxinus*, based upon speed of injection (Priestley *et al.* 1935), gave results which suggest that the vessels may be longer than the tree itself.

(b) *Laburnum vulgare J. Presl.*

Vessel segments which are apparently vessel terminations have been found in the spring wood of the trunk. These are of two types, in some the segment appears to be the termination of a single vessel (Pl. IX, fig. 5), whilst others have two perforations at the same end of the segment and, like those in *Fraxinus*, possibly represent common terminations linking two vessels. The arrangement of the

spring vessels in *Laburnum* in transverse section is such that vessels frequently occur in radial pairs as in *Fraxinus*, and in this case in tangential pairs also. From the relative frequency of the occurrence of the segments regarded as vessel terminations, it seems reasonable to regard the rarity of such segments in ring porous and their comparative frequency in diffuse porous types as evidence for greater vessel lengths in the ring porous.

The vessel terminations have always been elements which have tapered to a point at one end and in no case has one been found in the form of a transverse plate.

INJECTION EXPERIMENTS

A. *Injections using a fine suspension of Indian ink*

Previous injections carried out in Leeds had been performed on living trees and had given results of the order of 20 ft. for ring porous and 2 ft. or slightly more for diffuse porous trees. It was recognized, however, that these injections had never reached a vessel termination.

To overcome this an attempt was made to apply the injecting fluid under pressure. Twigs of *Acer* were cut and decorticated under the ink suspension and were then fitted to an apparatus by means of which ink under a pressure of $1\frac{1}{2}$ atmospheres could be applied to the base of the twig. When the apex of the twig was cut off, bleeding occurred for a short time and then ceased. After injection for 12 hours, maceration of the ends of the injections in 5 per cent chromic acid showed that the ink had in no case reached the end of a vessel.

The results of this method of injection were then considered in relation to the various postulations which have been made with regard to vessel contents. One conception is that put forward by Holle (1915) and Bode (1923) that the tracheal contents are water alone, and this may be considered with that of Scheit (1884-86), who suggested that tracheae contain water and water vapour. If either of these were correct, application of ink under pressure to the base of a twig should, on removal of the apex, force water out of the vessels and the ink would be expected to reach a vessel termination. In the case of Scheit's postulation the condition is essentially the same, since a saturated vapour does not obey Boyle's Law.

A third conception is that of von Höhnel (1879-81) and Schwen-dener (1886), who suggest that the tracheal contents are water, water vapour and air under reduced pressure. Considering this in

relation to the present problem, it is reasonable to assume that, when vessels fill up with water during winter, any air present in them will accumulate at that end nearest to the terminal bud. In this case, the application of ink under pressure to the proximal end of a twig, the apex of which had been removed, would only cause the ink to move into the vessels so long as some water and uncondensed water vapour remained. If, on the other hand, the twig was similarly cut under the fluid and was injected from the distal end, the majority of the open vessels would be likely to have had the end containing air removed; in such cases it should be possible to force the suspension to the vessel end, provided that along the file of vessels in this particular trachea no vessel shorter than the open vessel were present (or the air bubble in such a short vessel would block all movement along this line, as already discussed in the Historical section).

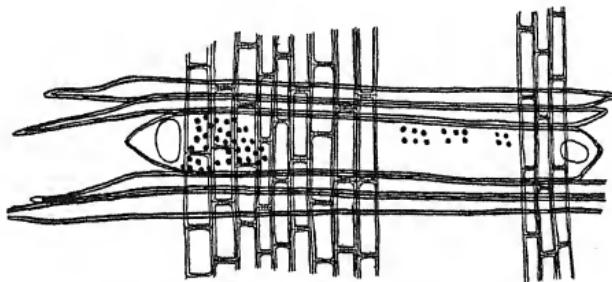
On testing this possibility by injection from the distal end, though the longer injections did not reach vessel ends, maceration showed without doubt that some of the injections which ended in the first inch of the twig from the end injected were vessel ends, in all respects similar to those already identified from normal macerated material (Pl. IX, fig. 6).

It was a matter of some surprise however that none of the longer vessels were injected to the end, as it seemed probable that some of the files at least would have only still longer vessels present beyond that which was cut and in process of injection. This fact led to a reconsideration of the suitability of the ink suspension for this purpose. It was shown experimentally that the sap electrolytes did not cause the coagulation of the suspension particles, yet transverse sections showed the injected vessels to be completely blocked by the suspension.

If a single vessel completely filled with water is considered, then, since the whole of the wall is permeable to water, the effect of forcing ink suspension into it will be to cause water to escape over the whole vessel surface (especially through the thin pit areas), whilst the contained carbon particles of the ink will be deposited on the wall. Owing to this lateral filtration, the suspension is only likely to reach the end in those open vessels with their terminations very near the point of injection before the lumen becomes blocked by particles. If the vessel had originally contained air as well as water, the filtration would be limited to that part of the vessel which had contained water, blocking would occur sooner and the injection distance would be less. This seems to be substantiated by the fact that

the longest injection lengths are always obtained nearest the xylem periphery and, as was stated by von Höhnel (1879-81), the water content is always higher in the outer rings.

Clear evidence for lateral filtration is seen in the appearance of macerated injected elements, where the particles of the suspension are aggregated in the pits of the vessel wall (Text-fig. 1). Where two vessels in contact with one another have been injected under the same pressure, no filtration takes place from one vessel to the other, so that pits on this wall remain free from deposit, whilst pits on the remaining portions of the vessel wall, where filtration to other types of element has been taking place, are rendered very conspicuous by the black deposit within them.



Text-fig. 1. *Acer Pseudo-Platanus* L. Termination of an injection with Indian ink suspension on a twig. The accumulation of particles in the pits is evidence that lateral filtration occurred during injection.

If the above experiments are carried out with a *Fraxinus* branch, about 4 ft. in length, the ink appears at the other end within a few seconds, so that evidently the vessels are longer than the piece of branch taken. The lack of resistance to the flow of the injecting fluid in such cases is associated with very little lateral filtration and little tendency for the vessels to become blocked.

The conclusions to be drawn from the Indian ink injections may be summarized as follows:

(a) They establish a qualitative, and perhaps a very rough quantitative, idea of the differences in vessel length between ring and diffuse porous types. This is especially true when the injections are performed on the tree at a time when the tensions in the vessels are at a maximum.

(b) Colloidal suspensions are not suitable as injecting fluids for the determination of accurate vessel lengths.

(c) If the injecting fluid is applied under pressure, lateral filtration occurs and the consequent blocking effects minimize the likelihood of injecting to the end any vessels that approximate to the maximum length for the given material.

N.B. In the experiments from which these conclusions were drawn, except those performed on the trunk as described under (a), the branches were cut from the trees as required over the period from November to February, but the majority of the experiments were carried out in February, when the water content would probably be at a maximum. The advantage of this will be obvious from the preceding discussion.

B. *Injection by the displacement of the sap in twigs by suspensions of Magdala red¹*

As the particles of the Magdala red suspension are smaller than the carbon particles of Indian ink, it was thought that this might be more suitable for injection purposes.

The method of injection in this case was to draw sap out of the tracheae at the older end of the twig, by putting it under reduced pressure, whilst the suspension was allowed to flow in at the other end to replace the extracted sap. The idea was based upon methods used by MacDougall (1903) and Bennett *et al.* (1927). The apparatus consisted of a tube with a side arm by means of which tension may be applied, and a capillary tube to indicate the amount of liquid that comes from the twig. The twigs were cut under water, decorticated at the older end and then placed in the apparatus. After some hours the twig was removed from the apparatus and was gradually cut back from the oldest end until the suspension could be seen in the vessels and so the injection length determined.

When the pressure for injection is induced by a tension at the opposite end of the twig, it may be raised as an objection that there will be leakage through the pith, and as a result the pressure may be reduced until it is insufficient to force the suspension to the end of the vessel. However, from radial longitudinal sections of *Acer*, it is seen that at each girdle scar region there extends across the pith a tissue of highly lignified cells, unaccompanied by air spaces, and this completely separates the pith of the extension growth of one year from that of the next, so that this criticism is probably not a serious one where a girdle scar region is included in the twig under injection.

¹ This dye was the "echt" or genuine Magdala red, which forms a fine suspension in water and not a true solution.

The maximum injection lengths obtained by this method were greater than those for comparable twigs by Indian ink injection, but as the red did not remain during maceration processes, it could not be seen whether the suspension had reached the end of the vessel. The longest injections occurred at the periphery of the wood and injection length was also found to increase with the age and diameter of the twig, as shown for *Acer* by the following results:

Average diameter of the injected part of the twig cm.	Injection length cm.
0·7	11
1·0	27
1·3	35

With *Fraxinus* and *Laburnum* twigs with a length of 4 ft., the suspension passed through in 20 sec., but the longer injections with diffuse porous types suggested that this suspension had some advantage over Indian ink.

C. Injections of vessels with molten paraffin wax

This method was devised so as to avoid if possible the disadvantages encountered with the use of colloids.

The most suitable wax was found to be a mixture of 90 per cent petroleum jelly with 10 per cent paraffin wax (M.P. 50–58° C.). This was stained with Sudan IV (Scharlach R) by placing solid dye in contact with the molten wax for some time.

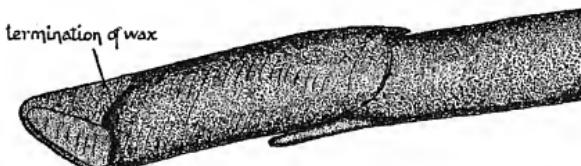
The twigs to be injected were dried for some weeks at a temperature of 120° F., so that it could reasonably be assumed that the vessels contained very little water. Ewart (1908) regarded methods involving heating or drying as dangerous on the grounds that the vessel walls become cracked, but in the present work the subsequent clean separation of the vessels on maceration showed that there had been no leakage of wax through any such cracks.

Twigs were used of *Acer* and *Fraxinus*, the dimensions of which were 48 cm. in length and 1½ cm. in diameter at the narrower end. After drying, the bark was removed over a distance of 2 in. at the youngest end. Whilst the twig was kept at a temperature above that of the injecting wax mixture, the vessels open at the distal end were evacuated by means of a water pump and the wax was allowed to flow into the evacuated vessels. Maceration of the ends of the injections showed that the wax had not reached even approximately near to the ends of the vessels (Text-fig. 2).

In the case of *Acer* some isolated vessels in the outer rings were completely injected through the entire 48 cm. of the length of the twig used, thus indicating that the maximum vessel length of twigs of that particular age may be greater than 48 cm.

In *Fraxinus* all the wax went through the vessels to the other end of the twig in a few minutes.

Comparing injection lengths obtained in Leeds on the living trunk of *Acer* in July 1932 with injection lengths on *Acer* twigs by the molten wax method, the results obtained were: maximum injection with ink, 27 in. (reckoning the injection above and below the cut); maximum injection with wax, 20 in. But it should be borne in mind that the wax injections were limited by the length of the twig and also, whilst the ink injections were performed on the trunk where the



Text-fig. 2. *Acer Pseudo-Platanus* L. Termination of an injection with wax by the low vacuum technique on a twig.

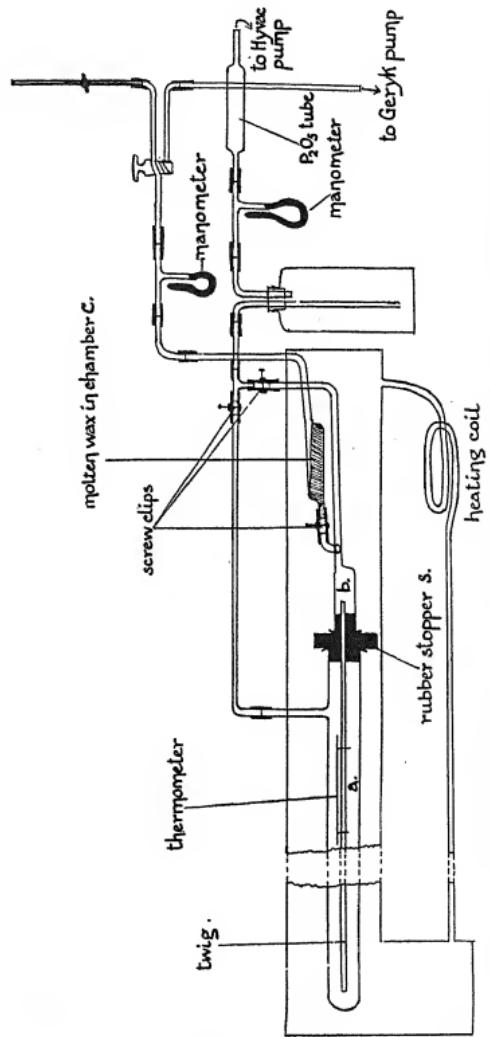
vessels are probably longest, the wax injections were from a 5- or 6-year-old twig.

Since vessels in wood were not completely filled by wax by this method, the behaviour of glass capillaries, sealed at one end and of equivalent dimensions to the vessels, was considered, both theoretically and experimentally. It was concluded that, under the conditions of the experiment on the twigs, there was no possibility of completely displacing the air in either the glass capillary or the vessel by wax.

The only method which seemed to offer any possibility of success was the use of high vacua.

D. Experiments on the injection with molten wax of vessels evacuated to a high vacuum

Twigs of *Acer*, about 5 ft. in length, were dried at 120° F. for 10 weeks and then placed in the apparatus shown in Text-fig. 3. The apparatus consists of a tube *a*, which contains the bulk of the twig and which is connected to the other part of the apparatus by the rubber stopper *s*, through which the decorticated distal end of the twig is passed. In the chambers *a* and *b* a high vacuum is produced



Text-fig. 3. Apparatus for high vacuum method of injection with molten wax. (For description see text.)

by a Cenco Hyvac pump, after warming up the apparatus for 4 hours at a temperature of 55° C. The vacuum connexion to *b* is then closed and the molten wax mixture (M.P. 42° C.) is allowed to flow from chamber *c* to chamber *b*. (The wax had previously been under the vacuum from a Geryk oil pump to remove dissolved air.) The vacuum above the wax and twig in chamber *b* was gradually released and the wax flowed into the vessels; meanwhile a high vacuum was maintained in the chamber *a*. After allowing the whole to cool gradually, the vacuum in chamber *a* was released and the twig removed and examined.

The terminations of injections were macerated; on microscopic examination it was seen that the injection had not reached a vessel termination, as may be seen from the end of this injection shown in Text-fig. 4.



Text-fig. 4. *Acer Pseudo-Platanus* L. Termination of an injection with wax by the high vacuum technique on a twig.

In the case of injection with Magdala red suspension on an *Acer* twig, 1 cm. in diameter, an injection length of 27 cm. was obtained; whilst by the present method injections of a similar twig, also 1 cm. in diameter, gave a length of 37 cm. Although this is not so long as the 48 cm. injection obtained with the low vacuum apparatus, it must be remembered that the twig in that case was older and 1½ cm. in diameter.

From considerations of the lengths of injection and the type of vessel segment in which the injection terminated, it would appear that this high vacuum technique had driven the injecting material nearer towards the terminations of the vessels than in the other methods employed.

During the injection it was noticed that, although there were no leaks in the apparatus, when the Hyvac pump was turned off there was a drop in the vacuum to about 0·2 mm.; this was quickly remedied when the pump was turned on again. Further, the vacuum in chamber *a* was allowed to stand, with the pump turned off, until the apparatus cooled off after the injection was completed, and during this time the vacuum first fell as mentioned before, but on cooling it increased again. This showed that during the injection, dry distillation with

associated production of vapours had been occurring in the twig as a consequence of the high temperature and high vacuum. These vapours condensed again on cooling so that the vacuum then increased again, but as the actual injection had been proceeding during the period of high temperature, it is probable that the presence of these vapours prevented the wax from penetrating to the actual vessel terminations.

E. *An experiment with coal gas*

Whilst all methods of injection so far attempted fail to make available accurate data for vessel length as the result of their complete injection by a visible suspension, yet all these methods have confirmed the previous Leeds work, which suggested a fundamental difference in length between the vessels of ring and diffuse porous woods. This result may be further confirmed by a simple if somewhat spectacular experiment. If the upper end of a severed branch system of a ring porous tree, such as *Fraxinus*, is firmly attached by means of stout rubber tubing to a cylinder of coal gas, the gas can readily be driven through a considerable length of stem and out through the vessels of the outermost annual rings at the butt end of the branch at a sufficient velocity to permit of its ignition.

In this way gas has been driven through an ash stem about 10 ft. in length. On the other hand in a diffuse porous tree such as *Acer*, when the tubing is attached to the distal end in the same way, the branch will have to be cut back to a length of approximately 2 ft., the exact length depending on the age of the twig, before gas is driven through in sufficient amount to permit of its ignition. In the case of the softwood, if the tubing is attached to the smallest possible length of stem, approximately $1\frac{1}{2}$ in., this is found to be quite sufficient to prevent any passage of the gas.

SUMMARY

1. By maceration methods vessel segments have been found in both ring and diffuse porous woods which look like vessel terminations; in some cases, when vessels have been injected with suspensions, similar segments have been seen to behave as such at the termination of the injection.

2. The method of sectioning cannot be relied upon to determine whether injection fluids have reached the end of a vessel. The only reliable method is to macerate the material, when the segment in question can be rolled over and viewed from every side.

3. The use of colloidal suspensions under pressure for purposes of injection cannot give reliable indications of vessel length as the vessels become blocked by the suspension; this is due primarily to accumulation of the suspended particles as lateral filtration takes place. In some cases this blocking effect is intensified by a retardation of the forward movement of the suspension owing to the presence of gas in the vessels.

4. Using a high vacuum technique on dried branches, with molten wax as the injection fluid, it still proved impossible to reach the ends of the vessels, though the wax appeared to reach very close to the ends. Failure to reach the end in this case is probably due to a slow distillation from the tissues under the conditions of temperature and vacuum necessary for the experiment.

5. All injection experiments confirm the view that there is a fundamental difference in vessel length between ring porous and diffuse porous types of wood. This conclusion may also be confirmed by a simple but striking experiment with coal gas under pressure.

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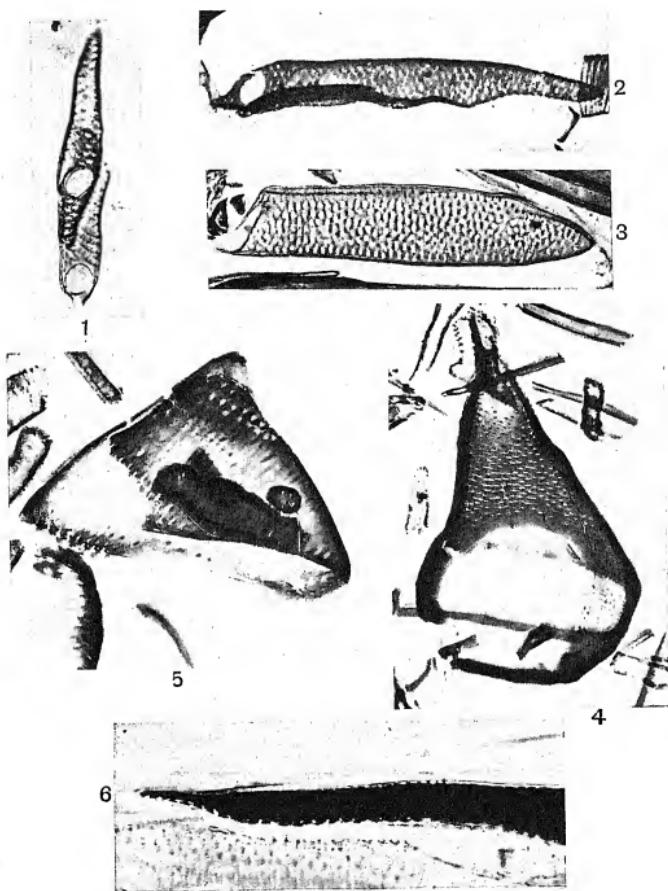
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HANDLEY—VESSEL TERMINATIONS



EXPLANATION OF PLATE IX

Fig. 1. *Aesculus Hippocastanum* L. Vessel termination from the end of the current year's extension growth. ($\times 270$.)

Fig. 2. *Aesculus Hippocastanum* L. Vessel termination from a 2-year-old twig. ($\times 190$.)

Fig. 3. *Aesculus Hippocastanum* L. Vessel termination from the trunk. ($\times 190$.)

Fig. 4. *Fraxinus elatior* L. Vessel termination from spring wood on the trunk. ($\times 200$.)

Fig. 5. *Laburnum vulgare* J. Presl. Vessel termination from the spring wood on the trunk. ($\times 290$.)

Fig. 6. *Acer Pseudo-Platanus* L. Vessel termination injected with Indian ink. ($\times 190$.)

Figs. 1-6 are all photographs of macerated material.

THE FRUITING OF MYXOMYCETES¹

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(With 1 figure in the text)

THE work of a number of investigators, notably that of Howard (1931 a), has made possible the laboratory culturing of myxomycete plasmodia. The cultures may be kept going uninterruptedly throughout the year, and thus, presumably, for an unlimited period of time. The method described by Howard involves simply boiling agar, oatmeal and water in the proportions, agar 15 g., oatmeal 30 g., water 1000 c.c., and pouring as sterile plates or test-tube slants. The plasmodium originally obtained from Dr Howard was that of *Physarum polycephalum*.

Cultures were kept growing in two Philadelphia laboratories throughout three winters and in one Paris laboratory for one summer. When culturing was interrupted, the plasmodia were allowed to form sclerotia by drying. From these sclerotia new cultures were made.

During a period of 2 years under varying conditions of moisture, temperature and light, no fruiting took place in any of the cultures. When a plasmodium was allowed to dry, it invariably formed a sclerotium. At the close of the 2-year period, cultures in two separate laboratories, coming from the same original sclerotium, fruited within a few days of each other. This occurred in early December. From then until the end of March, covering a period of nearly 4 months, the majority of the cultures fruited regularly and prolifically, always about 2 weeks from the time of culturing. While some few cultures failed to fruit, though growing under identical conditions, other members of the same series fruited in due time. After 1 April no more fruiting occurred, except for several sporadic cases, until near the end of June when nine out of ten cultures which were started at the same time formed sporangia simultaneously, 2 weeks from the date of culturing. This event closed the 2½-year period of cultivation upon which this article is based.

¹ Part of this investigation was supported by a grant from the Committee on Radiation of the National Research Council for which the authors are grateful. The senior author wishes also to express his indebtedness to Dr Pierre Allorge, Director of the Cryptogamic Laboratory of the Jardin des Plantes, Paris, for many courtesies.

While the external environmental factors which might be responsible for the fruiting of the myxomycetes were being studied, the time of fruiting reckoned from the day of culturing was noted in all cases and was found to be remarkably constant. The cultures in one laboratory were grown in light while those in the other laboratory were grown in darkness. The time of vegetative growth of the cultures grown in light averaged 14 days and the time of the other series grown in darkness averaged 17 days. Among the former only one culture fruited in 9 days after transplanting and one in 18 days; none in 10 days and none in 17 days; the rest all came within 11-16 days, the total averaging 14 days. While the average was 14 days, the greater number of cultures fruited in 16 days. Those cultures grown in the dark had a slightly longer period of growth with a minimum of 12 days, a maximum of 23, and an average of 17 days. The average of the entire lot was 16 days.

The foregoing facts were established in control cultures while other series were being run under different environmental conditions in an attempt to disturb the rhythm, by hastening, retarding, or stopping fruiting. Nine environmental factors were considered: dryness, depletion of nutrition, character of nutrition and substratum, temperature, light, poisons, acidity, radiation, and injury.

Fruiting has often been attributed to the tendency of plants to save themselves from extinction as a result of dryness. The hypothesis does not always hold (Seifriz, 1923), yet it might well prove true that myxomycetes sporulate because of excessive dryness. However, in our cultures, they responded to dryness not by fruiting but by forming scleridia. A naturally dehydrated plasmodium becomes exceedingly hard, breaking like a thin piece of wood, but when given moisture and a suitable substratum, flows and grows as actively as before. Scleridia several years old readily resume new growth. De Bary (1887) tells of a case, reported by Léveillé, of a sclerotium which grew after 20 years of dormancy. Evidence that lack of moisture is not necessarily a significant factor in fruiting is had from the fact that fruiting occurred regularly in cultures enclosed in glass chambers with a water-saturated atmosphere. Even more convincing was the following observation. Small culture dishes were kept in a large moist chamber on the floor of which was a 1-in. layer of water. It frequently happened that the protoplasm from cultures in the smaller dishes crept upon the surface of the surrounding moat of water, often covering, in the form of a fine net, many square inches (30 or 40) of water surface. On several occasions such a floating plasmodium

produced a large number of pendant strands up to 1 in. in length, which hung down within the water. At times the plasmodium would grow on the bottom of the glass chamber covered by the water. Such cultures fruited normally. The sporangia were held up in the air by their stalks distributed in rows along strands of protoplasm floating upon the surface of the water (Fig. 1). It is thus evident that absence of moisture is not necessarily a factor in the fruiting of myxomycetes.



Fig. 1. *Physarum polycephalum* fruiting on surface of water.

Depletion of nutrition is an environmental factor to which fruiting in culture is likely to be attributed. This can hardly be said of fruiting in nature where a rotted log would seem to supply sufficient nutrition for a very long time. Bits of plasmodium transferred to glass coverslips or to filter paper do not necessarily fruit, though their supply of nutrition is reduced to that within the protoplasm at the time of transference. While it is true that fruiting is always avoided if bits of the cultures are transferred to fresh agar-oatmeal plates, yet transplanting not only supplies fresh nutrition but it also eliminates toxic substances which have accumulated. Furthermore, failure to supply a fresh substratum does not always result in fruiting; it may simply cause death or the formation of a sclerotium.

The character of the substratum likewise appears to be unimportant, for spreading and fruiting take place on agar, water, glass, and paper without any evidence of acceleration or retardation.

Observations on temperature also gave negative results in that a variation from 15 to 38° C. produced no observable effect on fruiting. The cultures are, however, very sensitive to high temperature; a temperature of 38° C. is sufficient to kill them within a day.

Light has been suggested as a possible factor favouring the formation of sporangia. Our evidence does not support this in full. All the cultures grown in one of our laboratories were kept in the light of a north window. These fruited prolifically and regularly. The Paris cultures were grown in subdued light and failed to fruit over a period of 2 months. The cultures in our second Philadelphia laboratory were kept in the dark. Fruiting among these continued regularly, once it had started. Several cultures were put in direct sunlight without the time of fruiting being affected. Several cultures among those accustomed to light were isolated and kept in the dark. These failed to fruit at the expected time, while those remaining in the light fruited regularly. A change from light to darkness seemed to disturb the rhythm.

One fairly consistent fact appears to establish a relationship between sporangia formation and light and darkness: the cultures almost invariably fruited overnight. Howard (1931b) and Miller (1898) refer to nocturnal fruiting as characteristic of myxomycetes in their natural state and in the laboratory. The fact that, in nature, plasmodia come to the surface and therefore to the light to fruit, could be interpreted as standing in opposition to nocturnal fruiting, but sporangia must be in the open for spore dispersal. The impetus which starts a plasmodium on the way to fruiting occurs before it reaches the light, i.e. the plasmodium "goes" to the surface to fruit, and does not fruit because it is at the surface and in the light. Darkness, therefore, rather than light appears to be favourable for sporulation.

Poisons play so great a part in animal tissue culture that one of the first prerequisites discovered for the continued successful culturing of animal tissues was the frequent washing of the cultures, thus freeing them of their own poisons. The toxicity of cellular secretions and waste products appears to be a contributing factor in ageing. Life cycles, fatigue rhythms, and like periodic phenomena are sometimes influenced, if not determined, by the poisonous by-products of metabolism. As already stated, subculturing prevents fruiting; this may

be due to fresh nutrition or simply to the elimination of toxic substances.

Acidity is another factor which possibly determines the act or time of fruiting. Changes in acidity which take place in plasmodia may be very great. Certain of these appear, however, to be the result and not the cause of fruiting. The yellow pigment of *P. polycephalum* has been found to be an excellent natural $\text{\textit{pH}}$ indicator (Seifriz & Zetzmann, 1935). In a neutral medium, the natural indicator is yellow, in an alkaline medium it is bright green, and in an acid medium it is deep reddish orange. Young, actively growing plasmodia of *P. polycephalum* are bright yellow. Older cultures become orange, and sclerotia (in our cultures) were invariably of a deep reddish orange colour. At the time of fruiting, the plasmodial strands were yellow-green. The agar in the neighbourhood of the fruiting bodies took on a green colour, probably because pigment from the plasmodium escaped into an acid substratum. The pigment responsible for the colour changes is believed to be the group known as flavones. In terms of acidity young growing (yellow) plasmodia have a $\text{\textit{pH}}$ of 6.2, indicating a relatively neutral condition; old plasmodia and sclerotia (orange) are acid with a $\text{\textit{pH}}$ of 3.5; and fruiting plasmodia, or strands remaining after spore-formation (yellow-green), are alkaline with a $\text{\textit{pH}}$ above 7.0. These conditions all appear to be results rather than causes of the activities with which they are associated (active growth, fruiting and sclerotium formation). Radium (γ radiations) had no noticeable effect on fruiting. The plasmodia were extraordinarily resistant (indifferent) to radium radiation. They "avoided" radium needles in the main, but at times tolerated the radium to a surprising extent by flowing directly on and over the needles for several hours. Sporulation in irradiated cultures took place in quite the usual way and at the usual time, even though the cultures were continuously exposed to five 12 mg. radium needles within the 3-in. culture dishes for 10 days.

Severe injury, like most conditions unfavourable to vegetative growth, often leads to reproduction in plants. No such influence was to be noticed in myxomycetes, for they were unusually resistant to ill-treatment. When small masses of plasmodium were thoroughly shaken in water for the purpose of extracting the pigment, and then filtered, bits left upon the filter paper continued to flow and later fruited normally.

Not one of the nine environmental factors considered can be regarded either as the sole or the primary factor responsible for the

sporulation of myxomycetes. To what, then, can spore-formation be attributed? It seems that myxomycetes follow, as do most living things, a more or less definite growth rhythm or life cycle, but that this periodicity may be disturbed in culture.

That the growth period between transplanting and fruiting is well established is indicated by the fact that transplants made from parents just before the latter sporulate also sporulate; for example, three parents of slightly different ages fruited on 7 March. All were subcultured on 6 March, the day before fruiting took place, at which time there was no superficial evidence whatever that the plasmodia would sporulate the following night. The transplants grew a little, showing that the power to carry on pure vegetative growth was still present, but all three fruited simultaneously with their parents during the night. They had been separated from the parent cultures too late, and the change from vegetative to reproductive protoplasm in the parents had progressed too far to be stopped by subculturing. This behaviour is analogous to that of higher plants, e.g. bamboos, in which the impulse toward fruiting, once under way in a parent, cannot be stopped by the transplanting of an isolated sprout (Seifriz, 1923).

The foregoing facts having been established, an attempt was made to ascertain whether or not other growers of myxomycetes had had similar experiences. A thorough survey of the literature failed to reveal any such experimental results. Personal correspondence brought from Dr G. W. Martin the statement that *P. melleum* growing in artificial culture in several different media and kept in different rooms fruited almost simultaneously after having remained in the plasmodial stage for some time.

In the course of our inquiries we were informed of certain successful means of causing myxomycetes to fruit at will within several days of culturing from plasmodia. In spite of our best efforts we have not been able to trace these claims to their source. Other experienced investigators report that they know of no method which will cause a myxomycete plasmodium to form spores at any desired time. But let us assume that this report is true, it still in no way changes our general conclusion. The classical experiments of Klebs taught that rhythm in plants (in this case, the winter's rest) is very readily disturbed in some cases, with difficulty in others, and resists all efforts to disturb it in yet others. However, even the readily disturbed rhythms are none the less real, for they are followed when the plants are left to themselves in their natural habitat. So it is with the myxomycetes here reported upon. They possess a definite rhythm

which, though an innate, heritable protoplasmic quality, requires certain specific, and as yet unknown, environmental conditions in order to express itself.

SUMMARY

Cultures of the slime-mould, *Physarum polycephalum*, kept growing on oatmeal agar by subculturing every 10 days, failed to fruit for 2 years, and then, in two separate laboratories, fruited regularly over a period of several months. The time of fruiting varied from 9 to 23 days after transplanting, with most of the cultures fruiting within 11-17 days, the average of the total being 16 days. The rhythm appears to be well marked, but may end as suddenly as it arises with no fruiting taking place for weeks or months. All possible factors which might be responsible for the fruiting—dryness, depletion of nutrition, character of the nutrition and of the substratum, temperature, light, poisons, acidity, radium radiation, and injury—were considered, and none was found to be responsible, with nutrition and toxic substances alone having a possible influence. The growth rhythm is believed to be a definite protoplasmic quality, which requires certain as yet unknown conditions in order to express itself.

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ON SOME "THIRD" CONCEPTIONS IN FLORAL MORPHOLOGY

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Der Fortschritt in der begrifflichen Klärung ist nicht weniger notwendig als der Fortschritt in der Tatsachenerkenntnis.

L. v. BERTALANFFY, *Theoretische Biologie*, 1 (1932).

AMONGST the most characteristic features of modern science are: first, a variety of contradictory views on the same problems, secondly, the conflict of these with views recently accepted, and thirdly, the extravagance of the new conceptions. "Nothing is reliable—everything is possible" (Dingler, 1926). "New sets of ideas contradicting the naïve world conception are arising in immeasurable variety and struggling for life, and it is impossible to foresee which of them will maintain their position" (Bertalanffy, 1928). These "unprecedented" theories usually exclude the familiar alternatives, for which reason Dr Burkamp (1930) gives them the name of "third conceptions".

In floral morphology there have been until recently the two opposing theories of the euanthium and the anthodium. During the last decade a radical revaluation of the nature of the flower has been taking place. A struggle has been going on between such dissimilar and in part irreconcilable morphological systems as, for example, the doctrines of Gregoire, Hagerup, Heintze, Saunders, Thomas and McLean Thompson.

Thus views on the problem of floral evolution, a problem of secondary importance in biology as a whole, but cardinal to the morphology and taxonomy of the angiosperms, are showing on a smaller scale the same developmental phases as are found in scientific thought in general.

The last two International Botanical Congresses did well to devote a good deal of attention to these new theories which are so much more significant in the development of the science than whole volumes of new but undigested material. The *New Phytologist*, too, in which Hallier's system first appeared, recognized the importance of the new views by publishing the interesting controversy between

Dr H. Hamshaw Thomas (1934, 1935a) and Prof. McLean Thompson (1934a).

There should be as many participants as possible in such discussions, and in any case the circle of opponents should not be limited to the authors of these new conceptions. This is why I venture to put forward the following comments on the two theories which have already attracted the attention of this *Journal*. A summary of these theories is deemed unnecessary.

The most characteristic features of Prof. McLean Thompson's morphology seem to be his scepticism as regards the part played by palaeobotany in solving the problem of the origin of the flower and flowering plants; his disregard of comparative morphology, in particular of vascular anatomy in adult structures; and finally his exceptional belief in ontogeny. Never, perhaps, since Schleiden's own time have his ancient slogans ("jede Hypothese, jede Induktion in der Botanik ist unbedingt zu verwerfen, welche nicht durch Entwicklungsgeschichte orientiert ist". "Nur die Entwicklungsgeschichte kann uns über die Pflanze das Verständnis ergeben", etc.) sounded more insistently.

Dr Thomas attempts to discredit Prof. McLean Thompson's theory by recalling the gross errors made by Schleiden in the problem of fertilization. But what is important is not these errors but the fact that Schleiden belongs entirely to the past, that his making the ontogenetic method predominant as the only "path to salvation" (Goebel's expression) and his theory of the axial nature of the placenta and inferior ovary had already lost credit towards the close of the nineteenth century. Unfortunately, Prof. McLean Thompson disregards all critical literature on the question, confining himself to a casual remark on van Tieghem's vascular method.

In spite of Prof. Thompson the palaeontological method is certainly superior to the ontogenetic method. The former gives a direct picture of undoubted historical changes in structures and sheds light upon the actual process of evolution. The second method is based upon the hypothesis of recapitulation which itself needs to be proved. Prof. Thompson's empiricism is self-assumed. His work is founded on a speculation, the biogenetic law.

If ontogeny, including that of the flower, should prove one continuous recapitulation, Prof. Thompson's conclusions will have to be admitted by everyone. But has recapitulation in ontogeny, including that of the flower, been proved as yet? Quite the contrary. An evaluation of the ontogenetic method was given as far back as 1884 by

Nägeli, who protested against this "mode". He showed that the study of ontogeny strengthens our conception of the present state of a structure but not necessarily of its past history, not, that is, of the way in which it became what it is to-day. It is by no means more important than the comparative morphology of adult structures. The ontogenetic method attracts us by its simplicity only, for a comparative morphological treatment demands "more knowledge, more labour, more reflexion" (Nägeli, 1884).

Dr Massart, the author of perhaps the most fundamental work on recapitulation in plants, came to the conclusion that plants change so easily that the ancestral characters quickly give place to those of newly acquired adaptation (Massart, 1894). The same conclusion was reached by Shull (1905), by Diels (1906) and other authors who have specially studied the biogenetic law with a view to estimating its applicability to botany. The opinion of one as careful and authoritative as Dr Diels is so typical that we quote it here *in extenso*: "Auf botanischen Gebiet hat es (the biogenetic law) nicht einmal immer heuristischen Wert und wer sich von ihm leiten lässt, wird höchstens dazu gelangen, die Bedürfnisse seiner Phantasie zu befriedigen."

According to such an experienced plant morphologist as Dr Velenovsky (1905 etc.), all data of ontogeny of organs should be entirely disregarded in comparative morphological investigations if errors and inaccuracies are to be avoided: and other writers state that the law of recapitulation is entirely inapplicable to plants (Reinke, 1920; Dewar, 1931, etc.).

These writers may have gone to extremes, but this needs to be demonstrated, and the very existence of such scepticism should have made Prof. Thompson more cautious than he actually is. He entirely disregards the known prevalence of neogenesis, coenogenesis, and phylembryogenesis in both the animal and plant kingdoms. He does not take into consideration that the ontogenetic method applied to floral characters often leads to results which are extremely paradoxical and irreconcilable with the results of other methods of investigation, and susceptible to contradictory interpretations (see for example the interpretation of the ovule by Schleiden (shoot), Thompson (sporangium) and Hagerup (megasporophyll); and of the "carpel" by Hagerup (leaf, not sporophyll), Goebel (sporophyll), Gregoire (an organ *sui generis*), Thomas (cupule), Thompson (axial emergences)).

It is, of course, possible that Prof. Thompson's conclusions from ontogenetic data can be supported by data from vascular anatomy or other sources. Van Tieghem, however, studied the floral anatomy

of the Musaceae, Cannaceae and Zingiberaceae (1875) and obtained results entirely different from those of Prof. Thompson. According to him the pistil is formed of real carpels which merge at their apices into styles and have become concrescent in their lower parts with the perianth leaves, thus forming the inferior ovary. Van Tieghem's interpretation, by the way, easily explains the opening of the capsule. In Prof. Thompson's view we have a paradoxical process: the axis produces regular longitudinal and loculicidal fissures. A faint analogy might perhaps be found with the Cactaceae, where the tips of the old stems split lengthwise.

The ontogeny of the gynoecium in Scitamineae agrees in essentials with that in Umbelliferae. A number of facts and considerations taken from morphology and anatomy, which I collected (1926) to refute the axial theory of epigyny in this case, may be referred to also. Later and more precise data on vascular anatomy confirming the phyllous nature of the ovary in this family were given by Jackson (1933). It is to be noted, incidentally, that interpretations of the flowers of Umbelliferae and of similar floral types as widely different as those of Decaisne, Naudin, Schleiden-Payer, Koehne-Schumann, etc., have all been based on ontogeny. In all we know as many as ten different theories of this type of the ovary. Dr Velenovsky was quite right to take advantage of this fact when ridiculing the ontogenetic method.

As regards the Ranunculaceae and similar types, the vascular anatomy and characters of the flower and fruit in general again favour the phyllous nature of the carpels and stamens (Smith, 1926; Brouland, 1935).

Of particular importance is the fact that where there is undoubtedly invagination of the receptacle and sinking of the megasporophylls in it, this may be detected by the vascular system (Rosaceae, cf. Jackson, 1934). This is not found in the Scitamineae.

Prof. Thompson disposed of vascular anatomy in a few words, ascribing all to "physiological" features. He shares this one-sided attitude with many German botanists. I will not give facts proving the conservative nature of anatomical structures in the vegetative organs: they are numerous enough and well known. We have indeed a whole Doctrine of Conservative Organs (Jeffrey, 1917). Such facts, however, are known about the flower as well. In the female scales of the Cupresoideae and Taxodioideae the vascular bundles of the covering scales and seed scales (of Hagerup's "flower") remain isolated although these scales have fused together. In the flowers of *Digitalis*

the bundle of the abortive stamen is preserved. In the ovary of *Hordeum* there are, besides the placental, three more rudimentary vascular bundles pointing to its ancestral components. In the ovary of various Ranunculaceae there are rudimentary bundles of abortive ovules (e.g. *Trollius*) and of whole placentae (e.g. *Ranunculus*). Other examples of similar rudiments are given, for example by Beauverie & Durand (1930).

Numerous examples of the remarkable "inertness" of the vascular system are found in the floral organs of the Liliaceae, where nervation depends hardly at all on the shape, size, etc., of the corresponding organs and is an established systematic character (Simonsohn, 1901). Glück's investigation (1919) proved that the nervation of the floral organs is of great importance in their homologization, as it reproduces the system of "veins" in the vegetative leaves or their respective parts—sheath, base of lamina, etc. This, too, speaks for the conservative character of the vascular system. A no less remarkable example is the flower of the Compositae, where in the "lower storey", i.e. in the wall of the inferior ovary, the number of bundles varies greatly, ranging from 2 to 10 (Vidal, 1900), and so does not coincide with the number of members of perianth and androecium in the upper storey.

One could give many more examples of the same kind. "Flowers which were highly modified, especially by reduction, often have proof of the changes which had taken place, by retention of evidence in their skeletal tissues of earlier structural conditions, although external evidence had disappeared" (Eames, 1931; Beauverie & Durand, 1930). This is why the application of the "skeletal" method of vascular anatomy in the solution of problems of flower morphology (the schools of van Tieghem and Eames) has given such striking results which are both lucid and credible and which agree with other data besides those of ontogeny. This by no means dictates a mechanical interpretation of the vascular structures, archaic as they usually are. On the contrary, one must consider the physiology of the "skeleton" and its new formations (cf. the vascular bundles in emergences, Goebel's leaves devoid of bundles, etc.).

It is true that Vidal (1900) by employing the vascular method with ontogenetic and other data came to conclusions entirely different from those of van Tieghem, Eames and others, and close to that of Schleiden. But with Vidal the following errors are evident. (1) There is no comparison of the vascular system of the inferior ovary with the stele of its pedicel and with the vascular system of comparable superior ovaries (an unnecessary limitation of the method of

comparative anatomy). (2) The possibility of radial adnations of the vascular bundles is ignored, although we are led to acknowledge their existence from the study of anatomical series (Eames, 1931, e.g. among the Bicornes). (3) Confirmatory anatomical evidence is ignored in undoubtedly invaginated receptacles (*Rosa*), absent in others which Vidal judges invaginated only from ontogeny.

Let us agree with the "general rule" of Grelo (1898) that the vascular bundle always either belongs to or is intended for an organ externally expressed and retaining its ground tissue as well. If so, in dealing with the ovary of the Scitamineae we should be right in putting the matter thus: what does the vascular system of its components (presumably adnate) show us with regard to its origin? And if it speaks against deductions from ontogeny, this must be taken into consideration.

In Germany "every morphologist with a critical approach", according to Troll (1932), repudiates the idea that the vascular system¹ could be the starting-point for morphological interpretations. This criticism is most authoritatively expressed by Goebel, although in reality he merely repeats Grelo.

According to Goebel (1933) and many others, the vascular bundles depend on the organs and not vice versa. In our opinion they are mutually connected and neither is "determined" by the other. Goebel professes not to know a single case where only the bundle is preserved and not the corresponding phyllome, even in its early stages.² In connations the organ disappears morphologically—entirely or partially. Nevertheless, in the limits of a synphyllome it is present not only as a vascular "skeleton", but of other tissues as well. It is, however, just the skeleton that is convenient for distinguishing the number and position of the synphyllome components. Thus with its seeming "disappearance" the organ may not disappear entirely. That is why a search for it in the anatomical sphere is quite legitimate, and it is not surprising that traces of it are to be found. As well as this a complete morphological and anatomical abortion of the organ is possible. Thus the absence of any traces of the organ does not yet prove its absence in the ancestral structure. But it would be going too far to ignore cases where the interpretation of the vascular system is quite possible.

¹ We should like to point out that Dr Troll himself, when convenient, still interprets structures on the characters of the system of vascular bundles (see, for example, Troll, 1935).

² This remark is evidently directed against Miss Saunders's theory of the solid carpel.

If the question is controversial as regards the gynaeceum, it cannot be denied that in the corolla or calyx, for example, concrescent members often retain their individuality in the vascular system in spite of the considerable or total loss (in a mature state) of this individuality externally in the synphyllome.

The second part of the objection, the retention of the vascular traces of organs only when they have a morphological existence in early ontogeny, has neither been proved nor is it generally valid. Whether or no the organ has been visible as a separate entity at the early ontogeny of the flower, if in the mature state it is morphologically indistinguishable, only its bundles remaining, we have the right to speak of anatomical conservatism and of "vascular" rudiments.

The interpretation of the flower of such monocotyledonous plants as the Scitamineae (Prof. Thompson's main material), associated with van Tieghem's name and analogical interpretations, has at least no less, and in our opinion even more right, to be acknowledged than Prof. Thompson's so-called "new" view. The systematic consequences of his floral morphology, as based on controversial principles, could be put aside. We must remark, however, that the origin of monocotyledons direct from the gymnosperms is accepted by other writers as well: Engler, Campbell, Schellenberg, Emberger, etc., but they have in view different ways of evolution from those which Prof. Thompson envisages.

One more remark about the connexion between Scitamineae and Bennettitales. Prof. Thompson is the first to depict, in a concrete way, the transformation of the one type of strobilus into the other. Although at the beginning of the twentieth century much was said of the possibility of the second group originating from the first; the transition in the flower (in the gynaeceum) was never made clear. This even applies to the theory of the late Dr Hallier on the evolution of the flower, which, according to a letter he wrote to the author, "was seasoned with Greek terms" by Arber and Parkin, and is often erroneously coupled with their names.¹ Thanks to Prof. Thompson it has become quite clear which by-ways must be taken in order to pass from the gynaeceum of the Bennettitales to that of the angiosperms! But they are evidently the summits of different branches.

Prof. Thompson says nothing of where the interseminal scales of the gynaeceum, that is, the rudimentary megasporangiophore according

¹ I should like to emphasize that Dr Hallier himself has, in his latest papers and in letters, given preference to tracing the angiosperms from Cycadales, *sensu lato*, rather than from Bennettitales.

to Harris (1933), disappeared during the transition of Bennettitales to Scitamineae, etc., and whether their homologues should be sought in the gynaeceum of the angiosperms. Bennettitales, as is well known, are themselves angiosperms. Accepting the formation of a crater in the torus, with the immersion of the megasporangiophores therein, we heap one angiospermy on the other, and this needs an ecological justification. Besides, we know of no tendency towards the formation of toral craters in Bennettitales.

And this is Prof. Thompson's "empiricism"! The interpreter of the flower has, after all, not been the flower itself, as he promised us (1933), but Prof. Thompson. The "limits of speculation" were indeed stated in his hypothesis, but these limits offer a wide scope of action to the absolutized formula of the biogenetic law. The hypothesis as yet is based on a *petitio principii*.

We turn now to Dr Thomas's conceptions. The fact that all higher plants have a common origin in no way guarantees that from top to bottom, in all the branchings of their genealogical tree, variations of the same structures are repeated and consequently that the same morphological conceptions are applicable. Telomes are only peculiar to the lowest of the vascular phyla (Psilophyta), and higher up, with several exceptions, are replaced by three organs. It is useless to look for the ligules of the Lycopida in a Horsetail, or the umbrella-like spore-producing members of the Articulatae in the Pteropsida, as these organs characterize only their own special lines of development. And so on. Presumably Dr Thomas needed his general unifying concept in order to have the right to interpret the angiospermous gynaeceum from the view-point of the morphology of the Caytoniales, his *cheval de bataille*. However varied Dr Thomas's morphological considerations may be, whether he strives to embrace other hypotheses wholly (Gregoire, 1931) or in part (Thompson), the stability of his hypothesis depends on whether or not we acknowledge Caytoniales and angiosperms to be successive links in one and the same phyletic line.

Some botanists, for example Dr Krishtofowitch (1934), believe the Caytoniales to be in no way related to the angiosperms and to be derivatives of the Marsiliales. This opinion is based on the similarity between their leaves and between the sporocarps of Marsiliales and the pistil-like organs of the Caytoniales. But the leaves of *Sagenopteris* may possibly not belong to the Caytoniales. Besides, the tracing of close relationships by the leaves, though it may be customary with formalistic palaeontologists, should not be relied upon by the phylogenist, for leaves often show convergences. The ovary-like organs are

but superficially similar to the sporocarps. The sporocarps of the Marsiliales are known to contain sporangia of two kinds which are thrust outwards when fertilization takes place; in other words, there is no trace of pistil, ovary, stigma, seed or fruit. The ovary-like organs of the Caytoniales contain only megasporangia, or rather ovules which turn into seeds, remaining all the time in the depth of the ovary or "fruit". The microspores are caught by the stigma and germinate in the micropyle which they reach from the stigma through special "micropylar-stigmatic" channels (Harris, 1933).

The dissimilarity in structure between the pistil-like organs of the Caytoniales and the free carpels of the angiosperms is also evident. Dr Thomas is right in stating that there is no closer approach to angiospermy, outside the angiosperms, than among the Caytoniales. But the differences too are very great. The male as well as the female spore-producing members are extremely hard to compare with the corresponding organs of the angiosperms, and it is not easy to imagine the derivation of the flower as a whole.¹ However, the artificial constructions of Dr Thomas are the best proof of this. Even if the Caytoniales are related to the flowering plants, which fact has not been proved and hardly can be proved, as there is no hope of finding a fossilized 8-nuclear female gametophyte with double fertilization, or a fossilized xenophyte, then in any case they can hardly be direct ancestors but must have an indirect affinity. Any attempt to attribute a pinnate female spore-producing member to the angiosperms would mean disputing facts; and Prof. Thompson's criticism on this point is justified.

In his first important work on Caytoniales Dr Thomas (1925) "after a careful study" stated that the female spore-producing member is a complicated megasporophyll, and the ovary-like elements are formed by its segments ("laminae of pinnae only"). In more recent works by the same writer these segments "turn into" cupules of an enigmatic origin. A cupule is an indefinite and mixed conception. For a closer approach to Caytoniales the carpels of the angiosperms also "changed" in Dr Thomas's eyes into cupular (in his vague sense of the word)² formations, despite their great similarity to leaves, which has long been acknowledged by nearly everyone.

As Dr Thomas does not support his opinion, one wonders whether it has not been prompted by the wish to lean upon the ontogenetic data of Dr Gregoire, with his determination of the carpels as organs

¹ See, for example, Kozo-Poljanski (1928), cf. Hirmer (1935).

² The cupules of the Pteridospermae seem to be of a telome nature.

sui generis and not leaves. If the treatment of the carpel of the angiosperms, as homologous with the entire spore-producing structure of the Caytoniales, is indeed erroneous, then it might well be acknowledged as a complex of cupules of an enigmatic morphological meaning, in so far as Dr Gregoire's considerations are acceptable. These will be considered later. For the present we shall limit ourselves to the following remarks. This writer's views are again unacceptable as they are based on a triple *petitio principii*. (1) He deliberately limits the conception of homology, affirming that only organs having a similar ontogeny are really homologous. In searching for homologues in vascular plants all characters must be considered, including, of course, definite structures as well. (2) He does not take into account the possible manifestation of neogeneses in ontogeny and their prevalence in plants. (3) He forgets that the flower is not a typical shoot any more, but a product of its historical development, i.e. that *a priori* the flower is not to be expected to repeat completely all the characters of the vegetative shoot. Where there is continual change one ought not only to draw such conclusions as "either this or that", but to have more of "both this and that": the flower is and is not a shoot. As we know, Goebel (1933) makes the following comment on Dr Gregoire's doctrine: "One can be a splendid cytologist and at the same time an utter stranger to morphology."

Dr Thomas has made a one-sided use of vascular anatomy. Cases where structures which have morphologically disappeared are retained in the vascular system (see above) are well known to us, as well as a great many facts proving the relative conservatism of anatomical structures in general. We have, however, a great many examples of epharmosis in the vascular system in accordance with new physiological demands. Dr Thomas's interpretation of a triple nervation of the *Caltha*-like carpels, etc., as an echo of bygone times might be refuted by the suggestion that the marginal nerves have undergone a powerful secondary development in relation to their function of supplying the placenta.

The emphasis laid on the importance of basal stigmas and styles has only recently appeared in Dr Thomas's writings. In 1931 his reconstruction of the ancestral type of carpel of the derivative of the female spore-producing structure of Caytoniales showed the usual terminal stigma (1931a). The only motive for attaching significance to basal styles and stigmas is the linking of angiosperms with Caytoniales, which have a basal stigma. But, to begin with, the origin of the first group from the second has itself been proved by a similar assumption

—a vicious circle; secondly, it is uncertain how far this topography of the stigma is characteristic of the Caytoniales, whether it is not one of a series of types in this slightly known group. This idea is confirmed by the fact that among the Caytoniales' ancestors the characters of the cupulae and the position of their apertures, the future stigmas, were different. The double stigma may be explained in a much simpler way by the closing of the two edges of one and the same carpel (Goebel and others). Transmitting tissue (*tela conductrix*) is known to be present not among all, but in many different angiosperms (Capus, 1879; Gueguen, 1900–2; Tschirch, 1919; and many others), and even among those, for example Liliaceae (Strasburger, 1884), which do not suggest any likeness to Caytoniales; and in general it may be interpreted as a *secondary* structure of adaptation. Dr Thomas does not take into account the additional existence of an "invisible" transmitting tissue of a chemical nature (Sachs, 1887, and others).

The systematic conclusions from Dr Thomas's doctrine on the flower are evidently not clear even to himself. His advice to place at the base of the phyla the families and genera which have certain separate characters considered by these writers to be ancient, breaks up the old system but gives no principles for the building up of a new one.

Dr Thomas entirely passes over the question of how structures with so many ovules, with parietal, marginal and diffuse placentation, arose from corresponding Caytonian structures. It remains obscure whether the primary carpels were two-celled, which follows from the morphological series constructed by Dr Thomas, and if so where among angiosperms such gynaecia could be found, apart from such secondary structures as "false" septa.

Dr Thomas's first paper on Caytoniales was remarkable for its reticence in speculation. His further works, on the other hand, are conspicuous for their tendency to make Caytoniales the key to the solution of the most important problems in the morphology and systematics of the angiosperms, and even to the reconstruction of all the "old" morphology into a "new" one. To consider this as a "transgression" from palaeobotany, as Prof. Thompson evidently does, is out of the question. Palaeobotany, when its rich possibilities are widely made use of, has always yielded brilliant morphogenetical and phylogenetical constructions (Scott, Seward, Tansley, Bower, Kidston, Lang, Kräuse, Weyland, Jeffrey, and others). In our case we have an over-estimation of the importance of Caytoniales in general and of their various characters in particular. The former evidently

results from the enthusiasm of the writer who discovered this group and as a result has gained a well-deserved renown. The latter, presumably, is a methodological licence.

According to the reviewer of the *Fortschritte der Botanik* (Troll, 1932), Dr Thomas's paper (1931 b) is an example of a "horrible vulgarization" in the treatment of morphological problems. This vulgarization, however, is the subject of a whole series of special discussions of two world congresses of botany. The following qualities in Dr Thomas's doctrine are noticeable. First of all, it has clearly demonstrated that the Caytoniales are far from the angiosperms, that they cannot yield anything more to their morphology and phylogeny, and secondly, that the vascular system in the gynoecium, together with the transmitting tissue, contain very many original and morphogenetically unelucidated structures deserving of study.

In the discussion Dr Thomas versus Prof. Thompson we see a duel not only between palaeontology and ontogeny; it is a case of a conflict of two different modes of scientific thought. While Prof. Thompson tries in words to be a careful empiricist, himself limiting his method and matter generalized, limiting his conclusions within the scope of the objects studied, actually renouncing the comparative method and the use of the data accumulated before him, and finally reverting to Schleiden—Dr Thomas passes from one auxiliary hypothesis to another, constantly drawing in fresh and more various matter, in the name of his "new" morphology; unfortunately, however, with a conspicuous absence of a real leading morphological idea.

The conclusions of both writers are equally unacceptable to us. But our sympathies are with Dr Thomas rather than with Prof. Thompson. The paths which the former has taken in his work seem the right ones and with him we move forward; while the one-sidedness of the latter, accompanied by a monopoly of a method long ago discredited, only "brings us back to Schleiden".

To sum up, we consider that neither Dr Thomas's nor Prof. Thompson's conception of the nature of the flower has any advantages as compared with the classical euanthium theory of Hallier. But we are far from insisting upon a permanent existence for this conception. It will certainly be replaced by something new, with a closer approach to the nature of things.

Denn Alles muss in Nichts zerfallen
Wenn es im Sein beharren will!
(GOETHE.)

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REVIEWS

Das Grosse Moos im Westschweizerischen Seelande und die Geschichte seiner Entstehung. By WERNER LUDI. Pp. 344, with 47 text-figs. and 13 plates. Heft 11, Veröffentlichungen des Geobotanischen Institutes Rübel in Zürich. Bern: Huber. 1935. 19·80 Swiss francs.

At the end of the last glaciation the Rhone glacier withdrawing from the Swiss plains left behind a great terminal moraine which dammed back a great lake, some 200 km. long and 15 km. wide, into which drained the rivers Broye, Thiéle, Areuse, Shüss and Aare. By erosion of the outflow the level of the lake lowered and huge deltas were built into it by the rivers, dividing it into the lakes of Neuchatel, Biel and Murten. The detritus spread by the Aare over the plain and between the lakes of Neuchatel and Biel became the Grosse Moos, a large bog not drained and made suitable for cultivation until 1868. The drainage has had exactly the effects shown in the English fenland, namely, progressive peat wastage and lowering of ground-level. In many places the ground-level has sunk by 1 m. Before drainage the bog was practically treeless, but this is attributed largely to human activity. It was an extensive fen (Flachmoor), bearing *Molinista*, *Schoeneta*, communities of grasses and rushes and reed-swamp in the wetter parts.

Dr Ludi here expresses the results of intensive research into the origin and history of the Grosse Moos. Detailed stratigraphy of the lake and bog deposits has been established by lines of borings, and the results have been linked with archaeological horizons which occur here in abundance. The lake of Neuchatel is the home of pile-dwellings of all ages from Late Neolithic to the Iron Age, and they afford direct evidence of former changes in lake-level. The methods of pollen analysis have been freely used to establish a correlation between changing lake-level, the development of the bog, forest history and archaeological horizons.

In the older post-Glacial time the bog showed remarkably uniform development. From the high lake-levels of the early pine phase gradual lowering followed erosion of the outfall of Lake Neuchatel, so that "Verlandung" was progressive, and the bog surface dried out, and became tree-grown in the Mesolithic hazel period. At the end of this time set in a period of strongly varying lake-level, and peat formation was renewed. These conditions lasted through the mixed-oak forest phase and the fir phase which together cover the Neolithic period. The rise of the fir to dominance in the woodlands is held to reflect the onset of a wetter and probably cooler climate, in which sudden phases of high rainfall cause high lake-levels. One such period occurred at the end of the hazel period, another in the mixed-oak forest phase, and a third in the fir phase. Between the two last was the drier period when the oldest Neolithic lake dwellings were built.

In the Bronze Age, which approximately corresponds with the beech phase of forest development, the lake-level was lower than at any time before or since, and the bog was extensively tree-grown, chiefly with oaks, but also with beech and fir. The upper peat layers weathered to loam and Bronze Age pile-dwellings were built far towards the middle of the lake. The low lake-levels are attributed in part to the change in course of the Aare, but the period is also held to have been drier than that preceding or following it, and this is given as explanation of the replacement of the fir by the beech in the general forest cover.

In the post-Bronze Age the beech was replaced by spruce and fir together, and the lake dwellings of Halstatt and La Tène Age show higher lake-levels once more. The general increase in wetness of the climate is attested by the disappearance of trees from the bog under renewed peat formation, as well as by the spread of spruce and fir. The last great flood period was in late La Tène times; since then the lake-level has risen slowly and spruce has dominated the woodland. The influence of man in cultivation, tree-cutting, and drainage has made itself increasingly felt, of course much more especially since the great drainage scheme of 1868-80.

It is by the accumulation and correlation of such detailed and critical studies as these that knowledge of post-Glacial European history is best advanced, and no botanist interested in that subject can afford to neglect Prof. Ludi's comprehensive book.

H. GODWIN

Botany. By J. BEN HILL, LEE O. OVERHOLTS and HENRY W. POPP.
Pp. 672, and 335 text-figures. McGraw Hill. 1936. 24s.

With the shift in emphasis to new parts of the subject, and the increasing volume of botanical research, it becomes increasingly difficult to write a general text-book of botany which will give all-round satisfaction. The difficulty should best be met by a combination of lecturers, such as the authors of this book, who are together responsible for a complete university course. Despite this, and the usual competence of illustration and format associated with McGraw Hill publications, the result is not outstanding.

The book is divided into two parts: I, Structure and Physiology of Seed Plants, and II, The Plant Groups. So far as can be seen by consulting the accounts of special groups familiar to the reviewer, the accounts of life cycles of cryptogams are usefully up-to-date. The history of *Puccinia graminis* is revealed in a complexity now very formidable to the elementary student, the corresponding detail in *Ectocarpus siliculosus* is omitted and slurred into general statements. Each of these types requires almost a full lecture for clear exposition, and university teachers must soon decide if students are still to be asked to memorize all the features of all the types it has been customary to teach.

In the section on plant groups a rather dogmatic outlook prevails, and the deep disputes of rival taxonomists are not revealed. The Amentiferae are simply stated to be reduced forms, and there is no mention of the Caytoniales, though many groups of fossil plants are described, and although the origin of the angiosperms is specifically discussed.

The plant physiology is marred by an outlook shown in the extracts below. "The cohesion theory explains the ascent of sap exclusively on a physical basis. It excludes the living cells along the water column from taking any part in the lifting of the sap, although it requires the cells at the top of the column to be living cells. It should also be remembered that the water reaches the xylem at the bottom of the column through the living cells of the root, even though these cells may take no part in actually causing the sap to rise. Consequently sap ascent takes place only in a living plant", and, "If a ring of bark is removed from the lower part of a cutting of a willow or privet and the cutting placed in water, roots will develop only above the girdle, showing that the food necessary for their development can be obtained only from the upper part of the stem", and, "The controlling influence which that factor exerts which occurs in minimum is referred to as the law of the minimum."

I feel that the course on which this book was based was probably better than the book—the task of getting so much critically and interestingly to paper has been too great for more than partial success. Nevertheless the book is a full store of standard and recent botanical information, and for information rather than for outlook it will be useful to students.

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